Mechanisms of development of multimorbidity in the elderly

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ABSTRACT In ageing populations many patients have multiple diseases characterised by acceleration of the normal ageing process. Better understanding of the signalling pathways and cellular events involved in ageing shows that these are characteristic of many chronic degenerative diseases, such as chronic obstructive pulmonary disease (COPD), chronic cardiovascular and metabolic diseases, and neurodegeneration. Common mechanisms have now been identified in these diseases, which show evidence of cellular senescence with telomere shortening, activation of PI3K–AKT–mTOR signalling, impaired autophagy, mitochondrial dysfunction, stem cell exhaustion, epigenetic changes, abnormal microRNA profiles, immunosenescence and low grade chronic inflammation ("inflammaging"). Many of these pathways are driven by chronic oxidative stress. There is also a reduction in anti-ageing molecules, such as sirtuins and Klotho, which further accelerates the ageing process. Understanding these molecular mechanisms has identified several novel therapeutic targets and several drugs have already been developed that may slow the ageing process, as well as lifestyle interventions, such as diet and physical activity. This indicates that in the future new treatment approaches may target the common pathways involved in multimorbidity and this area of research should be given high priority. Thus, COPD should be considered as a component of multimorbidity and common disease pathways, particularly accelerated ageing, should be targeted.

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Multimorbidities share many common underlying mechanisms that may be linked to common causes such as oxidative stress http://ow.ly/H3siC

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Introduction

Non-communicable chronic diseases now constitute the greatest present and future medical burden in developed countries and are predicted to increase inexorably as populations age. This poses an enormous societal challenge, as it will incur increasing medical expenditure and even greater costs to society. As many elderly patients have multiple comorbidities this also complicates medical management. In the future specialists that are only knowledgeable about diseases of one organ will become less useful than doctors who understand multimorbidities, such as general practitioners and geriatricians. General practice databases indicate that almost 25% of the population have multiple morbidities and that this proportion increases substantially with age, so that most people over the age of 65 years have multimorbidity [1]. Multimorbidity is also linked to social deprivation and poverty. Disease management is, therefore, complicated by the fact that multiple treatments may be needed to deal with the different diseases and some of these therapies may interact with one another detrimentally. This suggests that it may be useful to explore common mechanisms between these chronic degenerative diseases, as it might be possible to develop new treatments that target common disease pathways.

Accelerated ageing appears to underlie many of the most prevalent chronic diseases, including ischaemic heart disease, osteoarthritis, osteoporosis, type 2 diabetes, metabolic syndrome, chronic renal disease and Alzheimer's disease, as well as chronic lung diseases, such as chronic obstructive pulmonary disease (COPD) and interstitial lung disease. Indeed, the most prevalent comorbidities in COPD patients such as ischaemic heart disease, diabetes, metabolic syndrome and metabolic bone disease may share common molecular pathways. In a cluster analysis of COPD patients age rather than disease severity accounted for most of the comorbidities [2]. One COPD phenotype is associated with cardiovascular and metabolic disease and is found in elderly patients [3]. A comprehensive analysis of comorbidities in COPD patients showed that almost all had at least one comorbidity and over 50% had four or more comorbidities [4]. Several distinct comorbidity clusters were identified, including one linked to cardiovascular disease and another linked to metabolic and cardiovascular disease.

All of this evidence strongly suggests that there may be common pathogenetic pathways linking these diseases. Identifying these pathways is important as this may lead to the development of useful biomarkers, but may also lead to novel therapeutic approaches (fig. 1). Indeed, existing treatments for a

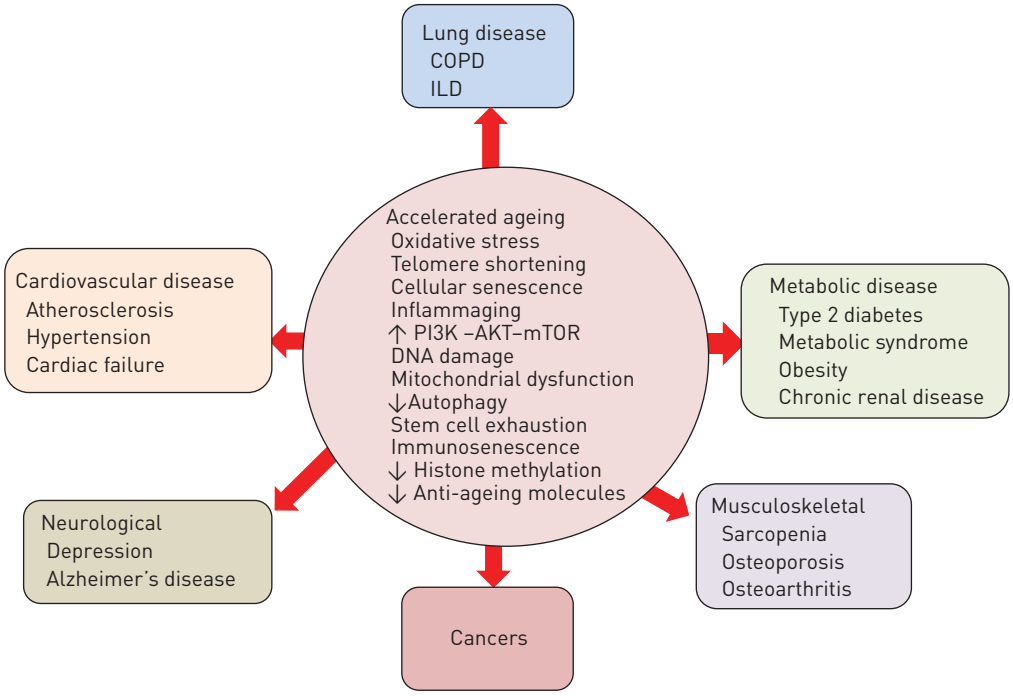

FIGURE 1 Several chronic diseases are associated with accelerated ageing, including chronic lung diseases, such as chronic obstructive pulmonary disease (COPD) and interstitial lung disease (ILD), cardiovascular diseases, metabolic diseases, musculoskeletal diseases, neurological diseases and cancers. Common mechanisms of accelerated ageing are shared between these diseases. PI3K: phosphoinositide-3-kinase; mTOR: mammalian target of rapamycin.

comorbidity may have some therapeutic value in COPD. For example, database studies have shown the use of statins that reduce mortality in cardiovascular disease, also appear to reduce mortality in patients with COPD [5]. Although this could be explained by a reduction in cardiovascular deaths among COPD patients there is also an apparent reduction in acute exacerbations, suggesting an effect on the lung disease [6], and also a reduction is associated lung cancers. This suggests that statins may be targeting pathogenetic pathways.

Telomere shortening

Telomeres are repeat nucleotide sequences (TTAGGG) at the ends of chromosomes that act as a protective cap and prevent loss of critical DNA and chromosome fusion during cell division. Telomeres are associated with a multiprotein complex called shelterin. Shortening occurs at each cellular division but is counteracted by telomerase. Telomerase is an enzyme complex that maintains telomere length and is made up of telomerase reverse transcriptase (TERT), an RNA template called telomerase RNA component (TERC), together with a protein dyskerin. In most cells telomerase activity is insufficient to maintain chromosome length and shortening of telomeres results in a finite number of cell divisions. A critical degree of telomere shortening leads to cellular senescence (replicative senescence) and cell death by apoptosis. Telomere shortening is a feature of normal ageing but is greater in diseases characterised by accelerated ageing, such as cardiovascular disease and diabetes [7–9]. Telomere shortening is found in circulating leukocytes in patients with COPD to a greater extent than in smokers with normal lung function and is particularly related to the risks of lung cancer [10–12]. Short telomeres appear to increase the risk of developing emphysema in smokers [11]. A large observational study in 45 000 Danish individuals showed that short telomeres in circulating leukocytes are associated with reduced lung function, although the independent effect of COPD once corrected for age and smoking, is relatively small. Shorter telomeres are also found in the alveolar epithelial and endothelial cells of patients with emphysema [13], although this is also seen in smokers with normal lung function [14]. Early onset emphysema has been described in a family with a genetic defect in telomerase activity [15]. However, the prevalence of telomerase polymorphisms among COPD patients has not yet been determined. Telomerase null mice with short telomeres showed increased susceptibility for development of emphysema after chronic cigarette exposure and there was a failure to downregulate p21, indicating cellular senescence [15]. Shorter telomeres are also reported in patients with idiopathic pulmonary fibrosis (IPF) and abnormalities in the telomerase components (TERT, TERC and dyskerin) are seen in familial IPF and occasionally in sporadic disease [16, 17]. Shorter telomeres are also found in IPF patients without any obvious abnormalities in the telomerase genes, suggesting that environmental factors such as cigarette smoking may contribute. It is likely that genetic determinants of telomere length may account for shared susceptibility to multimorbidities and may provide a mechanisms linking COPD with cardiovascular and metabolic diseases.

The mechanisms leading to telomere shortening in association with chronic diseases such as COPD and atherosclerosis are not yet completely understood. Increased oxidative stress is known to impair telomerase activity and to directly lead to telomere shortening. Telomere shortening in turn results in activation of p21, resulting in cellular senescence and the release of proinflammatory mediators, such as interleukin (IL)-6 and CXCL8 (IL-8). In cultured pulmonary endothelial cells from COPD patients there is reduced telomerase activity, which is associated with shorter telomeres, and with increased p21 and cellular senescence compared with cells from the lungs of age-matched nonsmoking control subjects [18]. There is also increased release of IL-6, CXCL1, CXCL8 and CCL2, indicating that the senescent cells are proinflammatory.

Cellular senescence

Diseases of accelerated ageing are characterised by enhanced cellular senescence, which is a state of irreversible cell cycle arrest (fig. 2). As discussed above, mammalian cells have a limited number of divisions, and then once DNA damage can no longer be repaired effectively, cells enter cellular senescence and subsequently undergo death by apoptosis. Cellular senescence involves the activation of the tumour suppression pathways p53 and p21 and the $p16^{INK4a}$/retinoblastoma protein pathways, which are activated by the DNA damage response in response to telomeric and non-telomeric DNA damage. The importance of an inability to repair double-stranded DNA breaks is highlighted by the rare Werner syndrome, a progeric disease that comes on in middle-age and mimics many of aspects of ageing, with atherosclerosis, diabetes and skin ageing occurring by the age of 40 years [19]. In this disease there is a mutation of a specific DNA helicase involved in DNA double-strand break repair that results in genomic instability.

Ageing should be regarded as a potentially protective mechanism to defend against the uncontrolled proliferation of cancer cells, but after procreation becomes potentially deleterious leading to loss of cell and organ function. Cellular senescence may be enhanced by extraneous stressful stimuli, such as oxidative stress and ultraviolet radiation in the case of skin. Unlike apoptotic cells, senescent cells remain metabolically active and therefore may influence other cells and exhibit what is termed a

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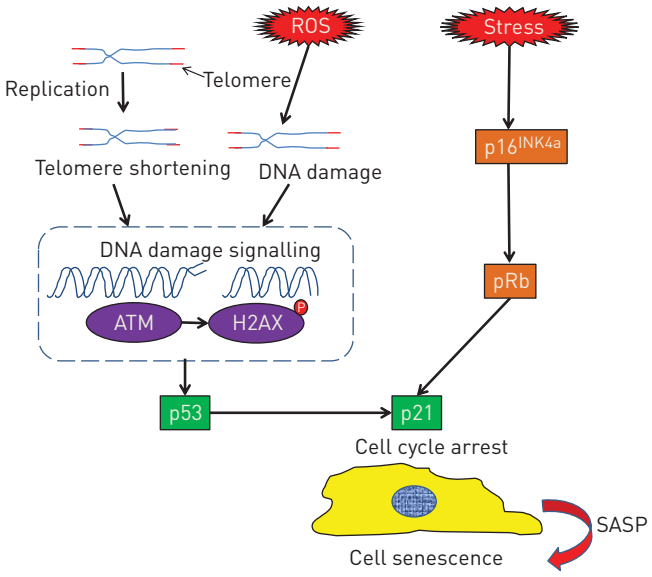

FIGURE 2 Pathways to cellular senescence. Repeated cell division leads to progressive telomere shortening (replicative senescence) or the DNA is damaged by reactive oxygen species (ROS), activating DNA damage pathways. These include the DNA repair kinase ATM (ataxia-telangiectasia mutated), which phosphorylates histone 2AX (H2AX), leading to activation of p53 and p21, which results in cell cycle arrest and senescence. Oxidative stress and other stresses activate $p16^{INK4a}$, which is a cyclin-dependent kinase inhibitor and phosphorylates retinoblastoma protein (pRb) which also activates the cyclin dependent kinase inhibitor p21. Senescent cells secrete cytokines and chemokines, known as the senescence-associated secretory phenotype (SASP), which amplifies and spreads cellular senescence.

"senescence-associated secretory phenotype" (SASP) [20, 21]. These cells secrete several inflammatory mediators that induce further senescence in the cell itself (autocrine) and in the surrounding cells (paracrine), thus amplifying and spreading cellular senescence. With advancing age, senescent cells accumulate in tissues and the SASP-elicited proinflammatory state ("inflammaging") is believed to have a complex influence on age-related conditions, such as COPD and cardiovascular disease. The SASP response is triggered by p21, which then activates p38 mitogen-activated protein kinase. This leads to activation of the pro-inflammatory transcription factor nuclear factor-κB (NF-κB), resulting in secretion of cytokines such as IL-6, growth factors such as transforming growth factor (TGF)-β and chemokines such as CXCL1, CXCL8 and CCL2, all of which are increased in diseases of accelerated ageing. CXCL8 acts *via* the chemokine receptor CXCR2, which has been shown to induce cellular senescence and DNA damage, whereas blocking CXCR2 reduces both replicative and stress-induced senescence [22]. Thus, the SASP inflammatory response itself mediates cellular senescence and thus spreads this response. Activation of the $p16^{INK4a}$ pathway activates NADPH oxidases, resulting in increased oxidative stress, which further activates NF-κB [23]. Removal of senescent cells that express $p16^{INK4a}$ using an inducible transgene delays the onset of ageing and prolongs life in an ageing mouse model, indicating that senescence itself drives further senescence [24]. This raises the possibility that strategies to eliminate senescent cells might be a therapeutic option in the future for diseases of accelerated ageing [25].

In emphysematous lungs cellular senescence is evidenced by decreased telomere length, enhanced expression of p21, p16 and p19, and senescence-associated β-galactosidase activity [26]. NF-κB is also activated in senescent cells, and drives inflammatory genes, such as cytokines and matrix metalloproteinases (MMPs), resulting in enhanced inflammation. Furthermore, in COPD there is an increase in serum concentrations of IL-6 and CXCL8 that are able to self-propagate senescence and, thus, maintain the phenotype by a feed-forward paracrine loop involving adjacent cells.

mTOR activation

There is increasing evidence that signalling though the phosphoinositide-3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) pathway is of critical importance in cellular senescence and ageing, and that inhibition of this pathway may extend the lifespan of many species [27]. Thus, rapamycin increases longevity in mice [28]. mTOR functions as two complexes, mTORC1 and mTORC2. The former plays the most important role in growth and is inhibited by rapamycin through its binding to the FK506-binding protein FKB12. This pathway is activated by oxidative and other cellular stresses and by nutrients, and results in

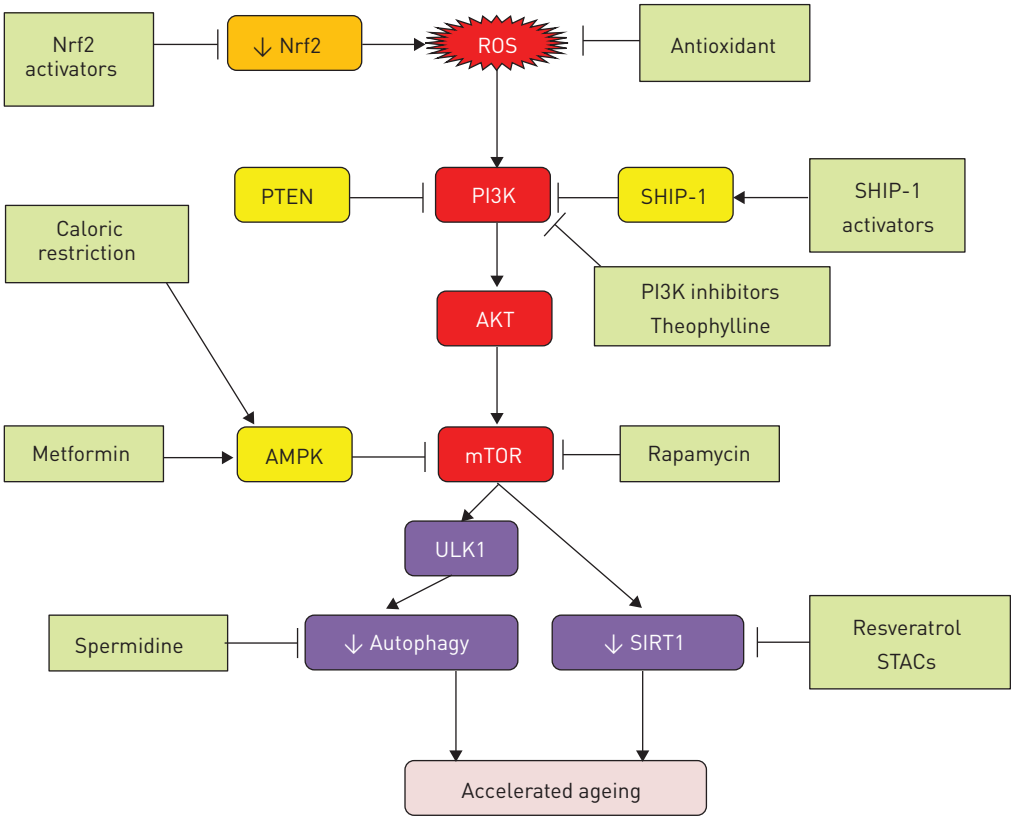

FIGURE 3 The phosphoinositide-3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) pathway is driven by reactive oxygen species (ROS), which activate PI3K. PI3K then, *via* activation of the kinase AKT, activates mTOR, which inhibits autophagy via unc-51-like autophagy activating kinase-1 (ULK1) and reduces sirtuin-1 (SIRT1) activity, both of which accelerate the ageing process. There are several endogenous and exogenous inhibitors of this pathway. Increased ROS may be the result of reduced activity of the antioxidant-regulated transcription factor Nrf2 (nuclear factor E2-related factor 2), which may be activated by small molecule Nrf2 activators. ROS may also be counteracted by antioxidants. PI3K is inhibited by the phosphatases PTEN (phosphatase and tensin homologue) and SHIP-1 (SH2-containing inositol-5′-phosphatase-1). SHIP-1 activators are now in development and there are several PI3K inhibitors including theophylline, which is selective for PI3Kδ. mTOR is inhibited by rapamycin and endogenously by 5′-AMP activated protein kinase (AMPK), which may be activated by caloric restriction and by metformin. SIRT1 may be activated by resveratrol and by novel SIRT1-activating compounds (STACs). Defective autophagy may be restored by the polyamine spermidine.

increased protein synthesis through ribosomal S6 kinases with secretion of growth factors, such as TGF-β and vascular endothelial growth factor. The mTOR pathway has multiple downstream effects that include inhibition of FOXO transcription factors, which are linked to longevity. There is evidence for PI3K activation in the lungs and cells of COPD patients as shown by increased expression of the downstream kinase phosphorylated AKT, which in turn activates mTOR (fig. 3) [29]. The PI3K pathway is inhibited by phosphatase and tensin homologue (PTEN) and SH2-containing inositol-5′-phosphatase-1 (SHIP-1), which are membrane tyrosine phosphatases. Both have oxidation-susceptible cysteine residues in the active site that are readily modified by oxidative stress, and thus, excessive oxidative stress reduces their activity. Indeed, PTEN polymorphisms are a genetic risk factor for COPD [30]. 5′-AMP activated protein kinase (AMPK) is activated by low ATP levels in the cell and inhibits mTOR activation. AMPK is activated by the biguanide metformin, which therefore inhibits mTOR signalling and extends the lifespan of several species, including mammals [31]. AMPK is activated by calorie restriction which has been shown to extend lifespan and also to reduce cardiovascular disease, neurodegenerative disease and diabetes in primates [32]. This suggests that activation of the mTOR pathway may play an important role in multimorbidity and inhibition of this pathway offers a future therapeutic opportunity [33].

Oxidative stress

Increased oxidative stress is the major mechanism that drives accelerated ageing though its damaging effect on DNA, activation of mTOR signalling and shortening of telomeres. Increased oxidative stress is well documented in the lungs of COPD patients, with increased biomarkers of oxidative stress in the

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breath [34–36]. This increase in oxidative stress is due not only to oxidants in cigarette smoke but also, more importantly, from activated inflammatory cells, such as neutrophils and macrophages, since oxidative stress persists in ex-smokers. Reactive oxygen species (ROS) are generated from NADPH oxidase enzymes (Nox1/3, 2, 4, 5 and Duox1/2) [37]. It is increasingly recognised that mitochondria are a major source of ROS and that this is linked to mitochondrial dysfunction in ageing cells [38]. Oxidative stress has several important consequences for the pathogenesis of COPD [39]. As discussed earlier, oxidative stress activates NF-κB, thus increasing inflammation. Superoxide anions interact with nitric oxide to form peroxynitrite which is increased in the lungs of COPD patients [40]. This leads to nitration of particular tyrosine residues on histone deacetylase-2 (HDAC2), resulting in its activation and degradation [41]. HDAC2 is markedly reduced in COPD [42] since there is another pathway activated by oxidative stress, PI3Kδ, which results in phosphorylation and degradation of HDAC2 [29]. The reduction in HDAC2 level leads to amplification of inflammation as HDAC2 is needed to deacetylate the hyperacetylated histones associated with activated inflammatory genes. It also results in corticosteroid resistance, since HDAC2 is the mechanism by which corticosteroids switch off activated inflammatory genes. Another consequence of oxidative stress is that proteins may be carbonylated and act as autoantigens to induce autoimmunity. Autoantibodies to carbonylated proteins are detectable in the plasma and lungs of patients with COPD [43].

Oxidative stress has long been recognised as a mechanism of premature ageing and the free radical theory of ageing has been influential [44]. An important mechanism linking oxidative stress to cellular senescence is oxidative damage of DNA. Smokers and COPD patients have an increase in 8-hydroxy-2′-deoxyguanosine (8-OH-2dG) concentrations in peripheral lung and type 2 pneumocytes compared with nonsmokers indicating oxidative damage of DNA [45, 46]. There is also an increase in the number of double-stranded DNA breaks demonstrated by an increase in phosphorylated histone 2AX (γH2AX) foci and the same cells also express p16, a marker of cellular senescence. Normally DNA strand breaks are efficiently repaired and in normal smokers there is an increase in the number of apurinic/apyrimidinic sites, indicating sites of base excision and repair. However, apurinic/apyrimidinic sites are not increased in COPD tissue, suggesting that there is a failure of the double-stranded DNA break repair machinery, which appears to be due to a deficiency in the DNA repair protein Ku86 [45]. This suggests that there is a defect in the DNA repair machinery in COPD patients and that this results in genomic instability and cellular senescence.

It is likely that oxidative stress is also a mechanism driving several COPD comorbidities, including atherosclerosis and diabetes. For example, serum 8-OH-2dG levels are significantly higher in patients with coronary artery disease [47] and serum concentrations are higher in patients with diabetes [48]. Increased lymphocyte and serum γH2AX levels were also linked to diseases of accelerated ageing, suggesting that oxidative DNA damage may be a common feature of these diseases [49].

Oxidative stress is normally counteracted by endogenous and dietary antioxidants. However, in COPD there is a deficient antioxidant response due to defective endogenous antioxidants and often compounded by dietary deficiencies of vitamins, such as ascorbic acid and tocopherol. There are many endogenous antioxidants that counteract oxidative stress that may be reduced in COPD. Extracellular superoxide dismutase (SOD3) is an important extracellular antioxidant in the lung and genetic polymorphisms associated with reduced activity have been associated with COPD [50]. Interestingly similar polymorphisms have also been linked to cardiovascular disease and hypertension, providing a genetic link between these diseases [51].

Most antioxidant genes are regulated by a key transcription factor, nuclear factor E2-related factor 2 (Nrf2), which regulates hundreds of antioxidant and cytoprotective genes [52]. Nrf2 is activated by oxidative stress through the disassociation of Nrf2 in the cytoplasm from associated proteins, such as KEAP1 and Cullin3, so that Nrf2 is free to translocate to the nucleus, where it binds to antioxidant response elements in the promoter regions of these genes. The resultant antioxidant response is an important means by which cells resist oxidative stress. However, in COPD there is a defect in activation of Nrf2 with defective expression of antioxidant genes in response to oxidative stress and cigarette smoke [53, 54]. This may be due to impaired transcriptional function of Nrf2 because of its acetylation as a result of reduced HDAC2 activity [54, 55]. Oxidative stress is increased in atheroma, hypertension and cardiac failure and this has been associated in several studies with a defective Nrf2 response [56]. In type 2 diabetes and metabolic syndrome there is defective function of Nrf2, which may promote increased oxidative stress and accelerated ageing [57]. Chronic renal disease is also characterised by increased oxidative stress and impaired Nrf2 responses [58]. The reason for defective Nrf2 function in diseases of premature ageing deserves further investigation and this may lead to novel therapeutic approaches to blunt oxidative stress and thus reduce cellular senescence.

Mitochondrial dysfunction

It is now recognised that mitochondria are an important intracellular source of ROS and a mechanism of oxidative stress. Mitochondria also regulate cellular homeostasis through membrane potential, making

acetyl CoA, and by their removal (mitophagy) [59]. Ageing is associated with the gradual accumulation of mutations in mitochondrial DNA with reduced resistance to oxidative stress [60]. Overexpression of catalase targeted to mitochondria extends lifespan in mice, suggesting ageing may involve mitochondrial function [61]. COPD is linked to increased mitochondrial ROS production, decreased intracellular antioxidants and reduced mitochondrial numbers [39, 59]. In addition, there is abnormal mitochondrial function in the skeletal muscle of COPD patients with muscle weakness, increased ROS production and decreased numbers [62]. Prohibitins (PHBs) are mainly localised to the inner membrane of mitochondria and appear to play a role in mitochondrial biogenesis and maintaining normal function [63]. PHB1 expression is reduced in the epithelial cells of smokers and to a greater extent in COPD patients, suggesting a mechanism for mitochondrial dysfunction in COPD patients [64]. Interestingly reduced PHB1 is highly expressed in endothelial cells and knock-down of this protein results in mitochondrial ROS production, cellular senescence and impaired migration and angiogenesis, suggesting a link to cardiovascular disease [65]. Furthermore, PHB1 is associated with diabetes and insulin resistance, and so may provide a mechanism that links multimorbidities to mitochondrial dysfunction [66]. Airway epithelial cells exposed to long-term cigarette smoke display mitochondrial fragmentation with increased expression of specific mitochondrial fission/fusion markers, oxidative phosphorylation proteins (complex II, III and V), and oxidative stress. Similar changes were found in epithelial cells from COPD patients and occurred to a greater extent than in cells from normal smokers, and these changes were accompanied by increased secretion of IL-1β, IL-6 and CXCL8 [67]. Furthermore, the mitochondrial stress marker Parkin and PTEN-induced protein kinase-1 (PINK1) were also increased in COPD patients [67, 68]. Knockdown of the PINK1 gene protects mice against mitochondrial oxidative stress induced by cigarette smoke [68]. Damaged mitochondria are normally removed by autophagy (mitophagy) and this involves highly regulated pathways, including PTEN, which may be reduced by oxidative stress and PINK1. Failure to remove dysfunctional mitochondria results in ROS generation and eventually cell death. Mitochondrial dysfunction and ROS generation during the ageing process may also activate the NLRP3 inflammasome, which generates IL-1β in chronic inflammatory diseases. The transcriptional regulator peroxisome proliferator activated receptor (PPAR)-γ co-activator (PGC)-1α is a critical regulator of mitochondrial biogenesis and generation of mitochondrial ROS. It is increased in the epithelial cells of mild COPD patients and then progressively reduced with increasing COPD severity [69]. Defective PGC-1α function has been linked to mitochondrial dysfunction and ageing related diseases, for example, skeletal muscle weakness, cardiac dysfunction, atherosclerosis, diabetes and metabolic syndrome [70, 71]. PPAR-γ activators, such as glitazones, may activate PGC-1α-regulated pathways to restore mitochondrial function.

Defective autophagy

To maintain normal function it is important for cells to remove degraded proteins, organelles (including mitochondria) and foreign organisms (such as bacteria), which they achieve by a highly regulated process termed autophagy. Defective autophagy is now seen as a feature of ageing and age-related diseases, including COPD [72, 73]. Autophagy selectively degrades and removes damaged proteins, organelles and pathogens *via* lysosomes. Ageing cells accumulate damaged and misfolded proteins through a functional decline in autophagy, leading to cellular senescence. Autophagy has been implicated in health and disease and, although autophagy is believed to play a protective role in response to exogenous stress, prolonged and excessive autophagy has been associated with cell death when the cytosol and organelles are destroyed irreparably. Inhibition of autophagy increases susceptibility to oxidative damage and apoptosis, whereas activation of autophagy leads to inhibition of apoptosis [74]. Cigarette smoke activates autophagy *in vitro* and *in vivo,* suggesting that autophagy is activated in order to degrade and digest damaged proteins and organelles. Alveolar macrophages from smokers show defective autophagy and this could contribute to accumulation of aggregates, abnormal mitochondrial function and defective clearance of bacteria [75]. Patients and mice with emphysema have increased markers of autophagy in lung tissue, as indicated by electron microscopic analysis and by increased activation of autophagic proteins, such as light chain-3, and autophagy may be contributory to apoptosis and alveolar destruction [76]. Other studies have demonstrated increased activation of the autophagic vacuoles (autophagosomes) in COPD. However, it is not clear if the completion of autophagy, as well as the initiation of autophagy, is taking place (a process termed autophagic flux). In other studies, macrophages from smokers have shown defective autophagic flux, resulting in accumulation of the substrate of autophagy, p62, and misfolded proteins due to dysfunctional lysosomal digestion of the autophagosomal load caused by a reduction in the lysosomal protein LAMP2 [75]. Inhibition of autophagy after cigarette smoke exposure enhances accumulation of p62 and ubiquitinated proteins in airway epithelial cells, resulting in increased cellular senescence and SASP with secretion of CXCL8, mimicking the changes seen in COPD cells [77]. Loss of autophagy may account for the reduction in mitophagy described earlier and also contribute to defective phagocytosis of bacteria in COPD [78]. Autophagy is impaired through the activation of PI3K–mTOR signalling, which results in inhibition of the unc-51-like autophagy activating kinase-1 (ULK1) complex that normally

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activates autophagy, thus linking defective autophagy to the accelerated ageing mechanisms discussed earlier [79]. For this reason defective autophagy also plays a role in other diseases of accelerated ageing, including cardiovascular and metabolic diseases, suggesting that it may be a common pathway in several degenerative diseases and could account for the clustering or comorbidities seen in COPD [80].

Stem cell exhaustion

During youth the process of cellular senescence is a beneficial compensatory response that contributes to clear tissues of damaged and potentially oncogenic cells [81]. This cellular checkpoint requires an efficient cell replacement system that involves clearance of senescent cells and mobilisation of progenitors to maintain cell numbers. In aged organisms, this turnover system may become inefficient or may exhaust the regenerative capacity of stem cells, eventually resulting in the accumulation of senescent cells that may aggravate the damage and contribute to ageing.

Damaged alveolar cells may be replaced by migrated progenitor cells [82]. However, once the alveolar architecture is destroyed, progenitors cannot rebuild the appropriate functional lung structure by themselves. Defective ability to repair tissue is associated with depletion of stem cells [81]. In mice, mutant mitochondrial DNA affects the quality and quantity of stem cells and interferes with the maintenance of the quiescent state, which is important for reconstitution capacity and long-term sustenance of somatic stem cells. Senescence of mesenchymal progenitor cells decreases regenerative potential, thus limiting the ability of the lung to repair in response to injury. Senescence of mesenchymal stem cells (fibroblasts and endothelial cells) could be a causative mechanism of emphysema and the failure to repair lung injury. The size of stem cell populations depends on the balance between self-renewal and cell differentiation. When the rate of self-renewal is higher than that of differentiation, the stem cell population expands, whereas when the self-renewal rate is lower than the rate of differentiation, the population declines as a result of exhaustion. In stem cells, ROS force the cells out of quiescence and into a more proliferative state by activating PI3K–AKT signalling and further promoting the production ROS, thus repressing the FOXO-mediated stress response and autophagy. Stem cell depletion may be important in COPD as persistent oxidative stress could force stem cells out of quiescence.

Type II alveolar epithelial cells are believed to be the progenitors of type I alveolar cells and show evidence of senescence in COPD patients [13]. Alveolar epithelial progenitor cells isolated from adult human lungs possess a phenotype characteristic of mesenchymal stem cells [83]. The transitional phenotype of alveolar epithelial progenitor cells between the epithelium and mesenchyme suggests that these cells may act as endogenous stem cells in lung tissue repair. Stem cells from human lung are c-Kit positive and may differentiate into epithelial cells and also into mesenchymal and endothelial lineages [84]. Indeed c-Kit, a tyrosine kinase receptor for stem cell factor, plays a key role in alveolar development and its deficiency leads to emphysema [85].

Circulating endothelial progenitor cells (EPCs) and specifically blood outgrowth endothelial cells are made in the bone marrow and are important for maintaining endothelial integrity [86]. EPCs from smokers and COPD patients show cellular senescence and increased DNA double-strand breaks compared with cells from nonsmokers and this is correlated with reduced expression of sirtuin-1 [87]. These senescent stem cells are poorly effective in repairing endothelial damage and provide a link between COPD and ischaemic heart disease. Many studies have documented dysfunction of EPCs in cardiovascular disease, including atherosclerosis, hypertension and cardiac failure [88]. EPCs are also dysfunctional in diabetes as a result of abnormal glucose metabolism and this may contribute to the high prevalence of cardiovascular disease in diabetic patients [89]. Renal pericytes play a role as local stem cells within the kidney and show signs of senescence as well as depletion in chronic kidney disease [90].

In diseases of accelerated ageing stem cells, including type 2 pneumocytes, EPCs and pericytes, all show features of cellular senescence, with DNA damage that may be the result of oxidative stress. This leads to loss of regenerative capacity and the eventual failure of organs, including lungs, heart, vascular system, nervous system, skeletal muscle, kidney, cochlea, lens and liver [91]. Understanding the molecular mechanisms of stem cell ageing is critical to elucidating the common mechanisms that underlie multimorbidity and offers the prospect of finding therapies to halt its progression or even to recover stem cell function [92]. Senescent stem cells characteristically have increased generation of intracellular ROS (mitochondrial ROS), mitochondrial damage, activation of the PI3K–mTOR pathway, DNA damage with double-stranded DNA breaks and a defect in the DNA damage-sensing kinase ATM (ataxia-telangiectasia mutated), defective proteostasis and a deficiency of anti-ageing molecules, such as sirtuin-1 (SIRT1) and FOXO transcription factors. For example, EPCs from COPD patients have activation of PI3K, reduced ATM and reduced SIRT1, all of which can be reversed by resveratrol, a sirtuin activator [87]. Accumulation of various types of DNA damage in stem cells with age is thought to be a major mechanism leading to the progressive inability of stem cells to repair organ damage and slowly progressive organ

failure [93]. Epigenetic changes in stem cells, including DNA methylation and histone acetylation, also play an important role in stem cell senescence, as discussed in the section on epigenetic mechanisms.

There is considerable interest in using stem cell transplantation to restore stem cells in ageing tissues [92]. Reprogramming of somatic cells into specific progenitor cells is a promising approach for the future. For example, induced pluripotent stem cells can be differentiated into myogenic cells to treat skeletal muscle weakness [94].

MicroRNAs and microparticles

MicroRNAs (miRNAs) are endogenous small noncoding single-stranded RNAs of ~22 nucleotides that regulate the post-transcriptional expression of hundreds of genes by inhibiting their translation or inducing degradation of targeted mRNAs. There is increasing evidence that miRNAs play an important role in disease pathogenesis and also in the ageing process. Thus, miRNAs regulate several key proteins involved in cellular senescence, such as p16 (miR-24), p53 (miR-885-5p), SASP (miR-146) and reduced SIRT1 expression (miR-34a) [95]. Genome-wide assessment of miRNA expression in monocytes of elderly compared with young individuals show that many miRNAs decrease with age and are implicated in regulating PI3K–mTOR signalling and DNA repair pathways [96]. The same miRNAs that have been implicated in cellular senescence are also found to be abnormal in diseases of accelerated ageing, such as atherosclerosis, Alzheimer's, diabetes and COPD [97]. Identification of key miRNAs involved in senescence may provide opportunities for therapy of multimorbidities in the future.

Microparticles (also known as microvesicles or exosomes) are small cell vesicles (0.1–1 μm) that may be released from cells, such as endothelial cells, epithelial cells and circulating blood cells, in response to cell activation, apoptosis and cellular stress, including oxidative stress. They contain proteins and RNA, including miRNA, and this provides a novel mechanism of cell-to-cell communication since the microparticles fuse with cell membranes to deliver their cargo into target cells. Microparticles from blood mononuclear cells were first shown to contain a variety of miRNAs, selected from the miRNAs in the cytoplasm, that could affect protein translation in target cells [98]. Several species of endothelial microparticles (differentiated by different surface markers and adhesion molecules) are increased in the circulation of patients with atherosclerosis, hypertension, metabolic syndrome and renal disease and are able to induce endothelial dysfunction [99]. Several types of endothelial microparticles are also increased in the circulation of COPD patients and may play a role in linking COPD to cardiovascular comorbidities [100, 101]. The increase in endothelial microparticles is related to disease severity and to the degree of emphysema, and may reflect the pulmonary capillary endothelial injury as the microparticles are positive for angiotensin-converting enzyme. There is an increase in circulating endothelial microparticles is smokers who have early emphysema [102]. Endothelial microparticles are further increased during exacerbations for over 4 weeks and could contribute to the increased cardiovascular risk following these events [100]. Microparticles are also derived from other cell types, such as epithelial and inflammatory cells, but their roles are less well defined. For example, cigarette smoke stimulates the release of microparticles that have proteolytic activity (MMP14) from macrophages *in vitro* [103]. Endothelial microparticles containing miRNAs are increased in age-related vascular diseases, including atherosclerosis, hypertension and congestive cardiac failure [104, 105]. It is possible the microparticles may carry miRNAs linked to promotion of cellular senescence and thus provide a mechanism for spreading the accelerated ageing process to different organs, including the kidneys and pancreas. The presence of distinct "ageing" specific microparticles might provide a biomarker of accelerated ageing in the future and eventually present a therapeutic target for novel therapies.

Epigenetic mechanisms

There is increasing evidence that genes have little effect on the ageing process. Epigenetic changes result in changes in gene expression that do not involve alterations in DNA structure and may account for long-term changes in gene expression. Epigenetic changes include DNA methylation, resulting in gene suppression, and modifications in histones (for example, by acetylation, methylation or phosphorylation), which lead to increases or decreases in gene transcription. Chromatin structure is determined by the relationship between DNA and associated histone proteins and is carefully regulated, but there is evidence that chromatin structure changes with age and becomes more "open", which means it is more transcriptionally active [106]. There is a general decline in histone proteins with ageing and consistent changes in histone modifications and associated modifying enzymes, with increased histone acetylation and either increased or decreased histone methylation. Changes in histone methylation with age are thought to be of particular importance in accelerated ageing [107]. In the rare Hutchinson–Gilford progeria syndrome, which involves accelerated ageing, there is upregulation of the "activating" histone mark trimethylated lysine (K)20 on histone H4 (H4K20me3), but downregulation of the "repressive" histone marks H3K9me3 and H3K27me3, resulting in decreased compaction of chromatin that is

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associated with increased gene expression. There is accumulating evidence that changes in histone methylation occur during ageing and regulate cellular senescence, although there is currently little information about the histone methylation status in diseases of accelerated ageing, such as COPD and cardiovascular disease. Histone methylation plays an important role in regulating the various proteins involved in autophagy, DNA repair and SASP. The role of different histone methyltransferases and demethylases in regulating cellular senescence is currently being intensively investigated, as selective inhibitors of these enzymes are in development. However, the role of histone methylation in diseases of accelerated ageing such as COPD and cardiovascular disease has hardly been explored.

The epigenetic changes associated with ageing are of particular relevance in stem cell populations and may result in dysfunction and depletion [108]. This was initially demonstrated in haematopoietic stem cells, with downregulation of genes linked to chromatin integrity and genomic stability, such as DNA methyltransferases, histone deacetylases and chromatin remodelling genes [109]. Mesenchymal stem cells from elderly people appear to have less potential for differentiating into different cell types and this is linked to changes in DNA methylation [110].

Immunosenescence

The immune system loses its efficacy during ageing, resulting in increased susceptibility to infection and chronic inflammatory disease, with an increased tendency to develop autoimmunity. Immunosenescence affects both innate and adaptive immunity leading to loss of function and has been implicated in multiple chronic diseases, thus providing a common contributory mechanism to account for multimorbidity. Ageing of innate immunity manifests as defective function in several cells involved in innate immunity, with impaired cell migration and signalling through pattern recognition receptors, such as Toll-like receptors [111]. Neutrophils show decreased phagocytosis, chemotaxis and apoptosis, whereas macrophages show defective phagocytosis and antigen presentation, and natural killer cells have a reduced cytolytic potential. Dendritic cells show reduced interferon production. There is a loss of Toll-like receptor function in dendritic cells, which is associated with decreased T-cell-mediated innate immunity, resulting in a reduced ability to fight pathogens in the elderly and an increased risk of carcinogenesis [112]. There is a loss of naïve T-cells and B-cells as a result of involution of the thymus with age as well as telomere shortening, with consequent reduced responses to new antigens. There is a decrease in ratio of $CD4^+$/$CD8^+$ cells and a loss of the co-stimulatory molecule CD28, with an increase in both $CD4^+CD28^{null}$ and $CD8^+CD28^{null}$ cells, which have reduced immune and vaccine responses [113]. For example, in COPD the result of this reduced immunity is a low grade chronic inflammatory response, which has been called "inflammaging" and has been implicated in the progression of several age-related diseases, including COPD, atherosclerosis, diabetes, osteoporosis and Alzheimer's disease.

One of the manifestations of immunosenescence is an increase in autoimmunity with increased production of autoantibodies, which may lead to further tissue damage. For example, in COPD there is evidence for increased autoantibodies directed against endothelial cells and against carbonylated proteins formed by exposure to oxidative stress in severe disease [43, 114, 115]. The increase in autoimmunity has been associated with an imbalance between Th17 and regulatory T-cells (T_{reg}). In COPD patients there is an increase in the ratio of Th17 to T_{reg} cells in sputum and in the circulation [116, 117]. A similar change in the ratio of Th17 and T_{reg} cells is seen in atherosclerotic plaques in atherosclerosis [118]. There is recent evidence that T_{reg} cells may even transform into Th17 cells under disease conditions with loss of the key T_{reg} transcription factor Foxp3 [119].

Little is known about the signalling pathways involved in immunosenescence. In ageing neutrophils the impaired chemotaxis response is associated with increased PI3K and restored by a PI3K inhibitor [120]. Increased PI3K–AKT–mTOR signalling is also found in autoimmune diabetes. Inhibition of PI3K prevents autoimmunity, suggesting a central role for mTOR signalling in immunosenescence [121] and this may be mediated *via* inhibition of the transcription factor Foxp3 resulting in reduced T_{reg} function [122].

Defective anti-ageing molecules and pathways

Several endogenous mechanisms to counteract the molecular changes of ageing have evolved and it is possible that these protective mechanisms may also become defective during the ageing process. Indeed, this has been proposed as an important contributory mechanism for accelerated ageing in COPD patients [123]. A lot of interest has focussed on the role of silent information regulator proteins, known as sirtuins, as anti-ageing molecules that regulate lifespan. Sirtuins are highly conserved NAD^+-dependent enzymes that play a role in resistance to stress, genomic stability and energy metabolism [124]. Of the seven sirtuins found in mammals most attention has focussed on SIRT1 and SIRT6 as both are related to prolongation of the lifespan in mammals. SIRT1 deacetylates many key regulatory proteins and transcription factors

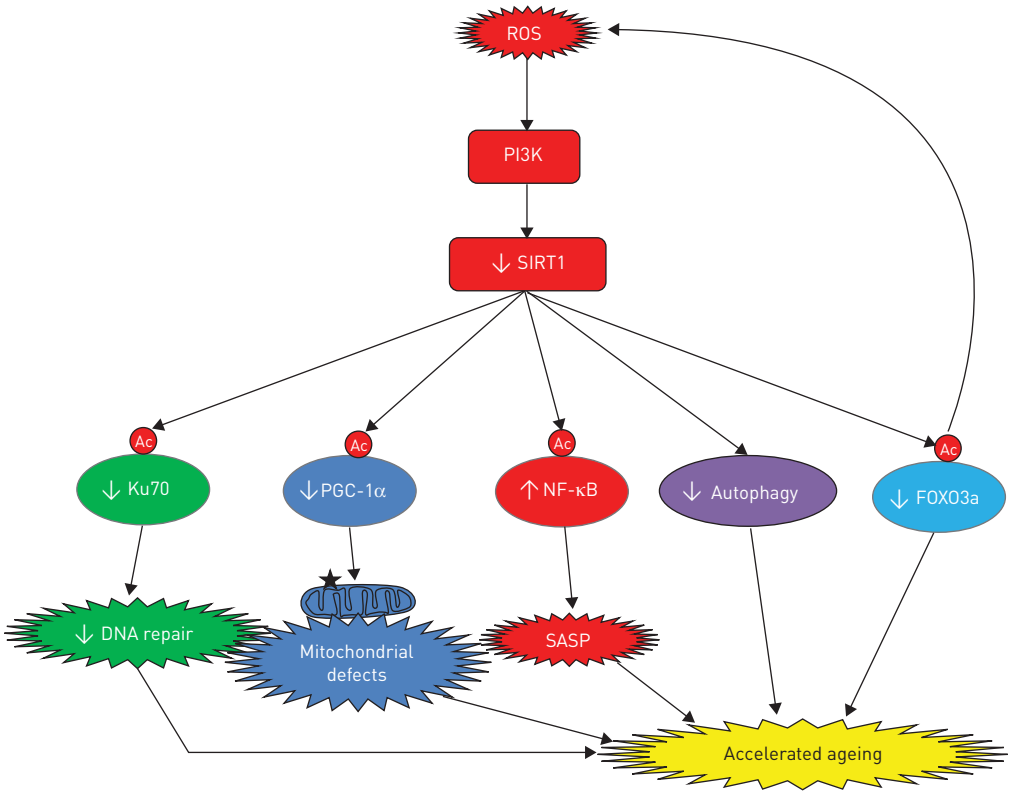

FIGURE 4 Sirtuin-1 (SIRT1) is reduced by reactive oxygen species (ROS) through the activation of phosphoinositide-3-kinase (PI3K) and this accelerates ageing through increased acetylation (Ac) of several proteins, including: Ku70, which is important in double-stranded DNA repair; peroxisome proliferator activated receptor-γ co-activator (PGC)-1α, resulting in mitochondrial dysfunction; nuclear factor-κB (NF-κB), which orchestrates the senescence-associated secretory phenotype (SASP); and forkhead transcription factor 3a (FOXO3a), which reduces antioxidants and further increases oxidative stress and inhibition of autophagy. All these actions contribute to accelerated ageing.

involved in DNA repair, inflammation, antioxidant gene expression and cellular senescence, including the PI3K–AKT–mTOR pathway and autophagy (fig. 4). SIRT1 binds to and deacetylates the transcription factor FOXO3a which enhances antioxidant responses, p53 which counteracts cellular senescence, NF-κB leading to suppression of inflammation, and PGC-1α which is important for normal mitochondrial function. SIRT1 is reduced in diseases of accelerated ageing, including COPD, atherosclerosis, cardiac failure, type 2 diabetes, metabolic syndrome, osteoporosis, chronic kidney disease and Alzheimer's disease [125, 126]. SIRT1 levels are reduced by oxidative stress *via* activation of the PI3K–AKT pathway and in turn SIRT1 inhibits mTOR signalling. SIRT1 also activates autophagy by inhibiting mTOR signalling [127]. The natural product resveratrol activates SIRT1 and has led to the development of more potent SIRT1 activating compounds, which are now in development for the treatment of age-related diseases [128]. SIRT6 is an ADP-ribosylase as well as a protein deacetylase and plays a key role in regulating DNA repair, telomere maintenance and metabolic homeostasis and, like SIRT1, is linked to extension of lifespan [129]. Reduced SIRT6 has been implicated in COPD, atherosclerosis, obesity and type 2 diabetes [125, 129]. Knockout of the SIRT6 gene results in a progeroid type of mouse showing features of accelerated ageing.

Animal models of accelerated ageing have identified other key molecules involved in senescence. The best studied is Klotho, which was found to be defective in a mouse model of premature ageing that has a substantially decreased lifespan, with atherosclerosis, emphysema, osteoporosis and insulin resistance, whereas Klotho overexpression extends lifespan [130]. Klotho is a transmembrane protein that is co-receptor of fibroblast growth factor (FGF23) and regulates insulin/insulin-like growth factor signalling, phosphate homeostasis, cell survival and proliferation. It appears to be protective against oxidative stress and decreased expression of Klotho is reported in chronic renal disease, diabetes, atherosclerosis, hypertension, osteoporosis and COPD. Senescence marker protein-30 (SMP30) is another anti-ageing molecule identified from a mouse model of ageing, which regulates calcium homeostasis and is sensitive to oxidative stress [131]. Expression of SMP30 is reduced in aged tissues, including the lung, and in SMP30

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knockout mice there is a marked increase in susceptibility to development of emphysema after exposure to cigarette smoke [132] and loss of cardiac protection [133].

Future targets for therapy

This review has identified several common molecular mechanisms that are abnormal in diseases of accelerated ageing that often coexist, suggesting that treatment directed towards restoring normal function could be valuable in treating several diseases that occur together as multimorbidity. This is an attractive therapeutic option for the future as targeting common pathways may make it easier to manage these diseases. While it is unrealistic to expect reversal of the normal ageing process (the elixir of life!), it may be possible to reduce the mechanisms that accelerate senescence in these diseases and several novel therapeutic targets that have been identified. Potential treatments for multimorbidity and accelerated ageing include drugs that reduce cellular senescence pathways, dietary interventions and lifestyle interventions, such as increased physical activity [134].

Pharmacological therapies

Better understanding of senescence pathways has identified several potential therapeutic targets that may prolong life and has led to drugs with the potential to inhibit accelerated ageing known as geroprotectors [135]. As discussed earlier, the PI3K–AKT–mTOR pathway plays a key role in cellular senescence and inhibition of autophagy, and inhibitors of this pathway may extend lifespan. Rapamycin, an antibiotic which is an immunosuppressive, is in current clinical use. It is a strong inducer of autophagy and extends the lifespan of all organisms, including mice [33, 136]. Unfortunately rapamycin and its analogues (rapalogs) have several adverse effects, including anaemia, pneumonitis and delayed wound healing, making it unsuitable for long-term use. Metformin, a biguanide, is widely used to treat type 2 diabetes and indirectly inhibits AMPK, resulting in inhibition of mTOR and extension of lifespan in mice, probably though increasing Nrf2-induced antioxidant gene expression [31, 137]. Since metformin is relatively well tolerated with chronic use it might be a suitable therapy for treating multimorbidity. PI3K signalling may also be inhibited by activators of the endogenous inhibitor SHIP-1 [138] and such drugs have already entered clinical trials for treatment of allergic disease [139]. Low concentrations of theophylline have been shown to inhibit oxidant-activated PI3Kδ, which may be involved in reduced SIRT1 levels after oxidative stress [29]. Long-term trials of low-dose theophylline are already underway in COPD patients and if successful this could be considered for other age-related diseases. Spermidine is a naturally occurring polyamine that triggers autophagy and extends lifespan, after oral administration, in several species, including mice and human immune cells, by reducing histone acetylation [140]. This suggests that spermidine or other polyamines might have therapeutic potential.

Resveratrol, a polyphenol found in red grapes and red wine, has been shown to increase lifespan in several species from worms to mice [141]. Its mechanism of action is disputed, but at least in part appears to be through the activation of SIRT1. A related compound quercitin, a flavone that is found in apples, has similar effects. However, resveratrol and related natural compounds, called stilbenes and flavones, have poor oral bioavailability, rapid metabolism and low potency. This has led to the development of novel potent synthetic analogues known as sirtuin activating compounds (STACs), which work *via* a common allosteric mechanism to stimulate SIRT1 activity [128]. Increasing SIRT1 activity has been shown to prolong lifespan and counteract a variety of age-related diseases, including neurodegeneration, cardiovascular disease, diabetes and cancer. In mice exposed to cigarette smoke the STAC SRT-2171 prevents the increase in MMP-9 that is associated with emphysema and improves lung function [125]. In addition, resveratrol reduces senescence in EPCs from COPD patients through an increase in SIRT1, indicating that exhausted stem cells may be an important target of STACs [87]. STACs are now entering clinical trials, initially for type 2 diabetes.

Oxidative stress appears to be an important mechanism leading to accelerated ageing, suggesting that effective antioxidants should, therefore, counteract the acceleration of ageing. Existing antioxidants, such as *N*-acetyl cysteine (NAC), are poorly effective as they are thiol derivatives that may be inactivated by oxidative stress, prompting a search for more effective, stable antioxidants. Novel antioxidants include nitrone spin-trap compounds and NADPH oxidase inhibitors, SOD mimetics and activators of Nrf2 [142]. Since there is evidence of mitochondrial oxidative stress in age-related diseases an intracellular antioxidant may be more useful. For example, the mitochondrial antioxidant SkQ1 reverses ageing-related biomarkers in rats, whereas *N*-acetyl cysteine is ineffective [143]. Nrf2 activators are of particular interest as Nrf2 may be defective in several diseases of accelerated ageing, including COPD, chronic kidney disease, neurodegeneration, cardiovascular disease and cancer [52, 144]. Sulforaphane, which occurs naturally in broccoli, is an Nrf2 activator but it is nonspecific and toxic in high concentrations, leading to a search for new drugs. Bardoxolone methyl is more potent but rather toxic in clinical studies, whereas dimethyl

fumarate (BG-12) has recently been approved for treatment of multiple sclerosis. Novel drugs that activate Nrf2 by interfering with the protein–protein interaction between Nrf2 and KEAP1 are in development [145].

Lifestyle interventions

Caloric restriction, which is the reduced intake of calories without malnutrition, prolongs the lifespan in species from yeasts to mammals, including primates [32]. This leads to inhibition of PI3K–AKT–mTOR signalling *via* activation of AMPK, and also reduces the release of insulin and insulin-like growth factor 1 and increases SIRT1 activity [146]. Caloric restriction protects against diabetes, cardiovascular disease, neurodegeneration and cancer. Several intermittent fasting regimes that give sufficient caloric restriction to activate anti-ageing pathways are now being explored [147]. Periodic fasting has been found to be effective in several animal models of age-related diseases, such as neurodegeneration, cardiovascular disease, metabolic syndrome and cancers.

The Mediterranean diet is rich in fruit, vegetables, red wine and olive oil, which contain flavones, polyphenols and stilbenes that may activate SIRT1; as a result the diet increases healthy life, with reduced incidence of neurodegenerative, cardiovascular and metabolic diseases and cancer [148]. For example, a Mediterranean diet may improve the function of EPCs from elderly patients and thus improve endothelial function in cardiovascular disease [149].

Physical inactivity is a risk factor for the development of diseases of ageing, such as COPD, cardiovascular disease and diabetes, and is an important determinant of mortality [150]. Aerobic exercise training provides significant clinical benefits in several age-related biomarkers, including lipid profiles, blood pressure, glucose tolerance, bone density, depression, loss of skeletal muscle (sarcopenia) and quality of life [151]. The benefits of pulmonary rehabilitation programmes in COPD are well established with improvements in lung function, reduced exacerbations and better quality of life [152].

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