Role of autophagy in lung diseases and ageing

Yan Zhang, Jin Zhang and Zhiling Fu

Department of Anesthesiology, Shengjing Hospital of China Medical University, Shenyang, China.

Corresponding author: Zhiling Fu (18940258057@163.com)

Shareable abstract (@ERSpublications)
Autophagy is a "housekeeping" survival strategy and its activity decreases with age. Manoeuvring autophagy can be a potential therapeutic target in ageing-related pulmonary diseases.
https://bit.ly/3URj2bN

Cite this article as: Zhang Y, Zhang J, Fu Z. Role of autophagy in lung diseases and ageing. *Eur Respir Rev* 2022; 31: 220134 [DOI: 10.1183/16000617.0134-2022].

Received: 20 July 2022
Accepted: 08 Nov 2022

Abstract

The lungs face ongoing chemical, mechanical, biological, immunological and xenobiotic stresses over a lifetime. Advancing age progressively impairs lung function. Autophagy is a "housekeeping" survival strategy involved in numerous physiological and pathological processes in all eukaryotic cells. Autophagic activity decreases with age in several species, whereas its basic activity extends throughout the lifespan of most animals. Dysregulation of autophagy has been proven to be closely related to the pathogenesis of several ageing-related pulmonary diseases. This review summarises the role of autophagy in the pathogenesis of pulmonary diseases associated with or occurring in the context of ageing, including acute lung injury, chronic obstructive pulmonary disease, asthma and pulmonary fibrosis, and describes its potential as a therapeutic target.

Introduction

The lungs are not only the primary location where gas exchange occurs in mammals but also have the largest epithelial surface area in the body; therefore, the lungs represent a unique interface with the outside environment and face ongoing chemical, mechanical, biological, immunological and xenobiotic stresses over a lifetime [1, 2].

Over the last century, due to improvements in living conditions and the continuous development of medical technology, the human lifespan has increased dramatically, and the population is ageing at a rate never seen before in human history [2, 3]. Age-related ailments represent a formidable global socioeconomic burden and a significant healthcare challenge [4, 5]. Advancing age progressively impairs lung function in otherwise healthy individuals; lung-resident cells rely on robust stress response pathways to stave off cumulative damage, yet in an ageing lung, the homeostatic control of wound healing following stress has an increased likelihood of being perturbed, elevating susceptibility to diseases. Therefore, many lung diseases are more prevalent and lethal in the elderly. Specifically, the recent coronavirus disease 2019 (COVID-19) pandemic, which further contributes to the global impact of lung diseases, highlights the increased susceptibility of the elderly to acute respiratory distress syndrome (ARDS) and subsequent fibrosis [6] and underscores whether there are age-related molecular determinants that can be targeted for therapeutic purposes to mitigate morbidity and mortality in elderly populations [7]. At the molecular and cellular levels, the nine hallmarks of ageing (genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication) are listed by López-Otín *et al.* [8] and are implicated in the pathogenesis of ageing-related lung diseases [9–11].

Autophagy is a "housekeeping" survival strategy involved in numerous physiological and pathological processes in all eukaryotic cells [12]. Autophagic activity decreases with age in several species, whereas the basic activity of autophagy extends throughout the lifespan in most animals, indicating that it is one of the convergent mechanisms of several longevity pathways [13]. Moreover, dysregulation of autophagy has been proven to be closely related to the pathogenesis of several pulmonary diseases.

In this review, we outline the mechanism of autophagy, focusing on its fundamental mechanisms in ageing and age-related pulmonary diseases, and discuss the therapeutic potential of regulating autophagy.

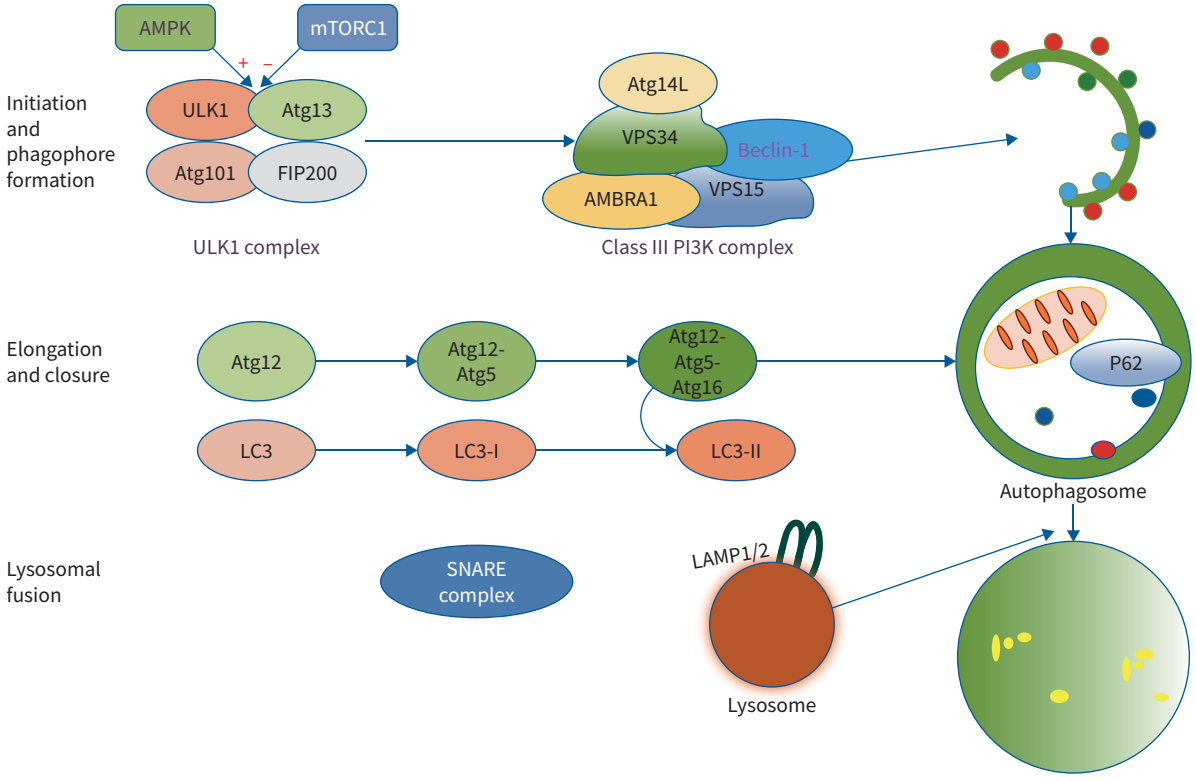

FIGURE 1 Illustration of autophagy. Autophagy involves the formation of autophagosomes and their fusion with lysosomes to form autolysosomes. The process is typically divided into distinct stages: initiation, elongation/closure and autophagosome–lysosome fusion. Initiation begins with activation of the complex. Unc-51-like autophagy activating kinase 1 (ULK1) and Atg13 are key to the ULK1 complex and are further supported by Atg101 and FAK family-interacting protein of 200 kDa (FIP200). Mammalian target of rapamycin complex 1 (mTORC1) binds to ULK1 and inhibits the ULK1 complex, and 5′ AMP-activated protein kinase (AMPK) phosphorylates mTORC1, resulting in the dissociation of mTORC1 from ULK. The ULK1 complex activates a class III PI3K complex consisting of VPS34, VPS15, Beclin-1 and Atg14L and activates the molecule in BECN1-regulated autophagy protein 1 (AMBRA1). The phagophore elongates and encloses to a double-membrane autophagosome. This step is tightly regulated *via* the ubiquitin-like conjugation systems. For example, the Atg12–Atg5:Atg16L1 complex conjugates phosphoethanolamine to LC3 to LC3-II, and LC3-II promotes substrate uptake upon binding to different receptors, such as p62. The autophagosome fuses with a lysosome to form an autolysosome for degradation. The inner content is released into the lysosome/autolysosome and is degraded by lysosomal hydrolases. The SNARE-like proteins may play critical roles in autophagosome–lysosome degradation. LAMP1: lysosomal-associated membrane protein 1.

Overview of autophagy

Three types of autophagy have been distinguished based on their differing mechanisms of cargo sequestration. In this study, we discuss the most studied form of autophagy: macroautophagy (hereafter simply referred to as autophagy). Autophagy involves the formation of autophagosomes (double-membraned vesicles) and their fusion with endosomes or lysosomes to form amphisomes or autolysosomes. Both the formation and turnover of the autophagosome involve evolutionarily conserved genes: autophagy-related (Atg) genes [14]. Autophagy is highly dynamic, and its process is typically divided into distinct stages: initiation, elongation/closure and autophagosome–lysosome fusion (figure 1).

Based on cargo specificity and delivery mechanisms, autophagy can be divided into two types: nonselective autophagy and selective autophagy (including mitophagy, pexophagy, endoplasmic reticulum (ER)-phagy, ribophagy and lipophagy).

Physiological role of autophagy and impact of autophagy on ageing and ageing-related pulmonary dysfunction

Role of autophagy in biology

Autophagy modulates various physiological processes in cells and plays a fundamental role in cellular, tissue and organismal homeostasis [12]. Therefore, the occurrence of the “diseased” state may be associated with autophagy dysregulation due to alterations in the central aspects of multicellular organism biology [15].

Autophagy is a particularly important regulatory mechanism in the lungs. Autophagy is abundant in lung epithelial cells. Epithelial autophagy is activated in the developing mouse lung through 5′ AMP-activated protein kinase (AMPK) activation, and the inhibition of AMPK-mediated autophagy reduced lung branching *in vitro*. The conditional deletion of Beclin-1 in mouse lung epithelial cells, at either early or late gestation, resulted in lethal respiratory distress at birth or shortly after birth [16].

Role of autophagy in ageing and lifespan

Autophagy activation has been proven to extend animal lifespan; it decreases with age in numerous species [17, 18]. The transcriptional and epigenetic regulation of Atg genes impacts the physiology and lifespan of animals [19, 20]. For example, mutations or loss of function in Atg1, Atg7, Atg18 and Bec-1 reduced the lifespan of *Caenorhabditis elegans* [21]. In contrast, the overexpression of Atg5 extended the lifespan of mice [22]. However, several investigations suggested that autophagy can be harmful under certain circumstances and in some models. For example, moderate overexpression of Atg1 substantially extends the lifespan of *Drosophila*; however, strong expression is toxic [23]. These observations may reflect the complexity of autophagy in ageing modulation. Nonetheless, we can infer that the maintenance of functional autophagy is essential for organismal ageing and that dysregulation of autophagy, whether insufficient or excessive, contributes to cellular deficits and functional organismal decline.

Crosstalk between autophagy, ER stress/unfolded protein response (UPR) and apoptosis

Besides autophagy, ER stress/UPR and apoptosis are two fundamental biological processes essential for manifold cellular functions in health and disease [24]. Alteration in the expression of apoptosis, autophagy and UPR markers is correlated with lung function in lung tissue [24].

The UPR system is controlled by three ER transmembrane proteins and 78 kDa glucose-regulated protein. Transmembrane proteins include inositol-requiring enzyme 1α (IRE1α), protein kinase RNA-like ER kinase (PERK) and acting transcription factor (ATF) 6 [25]. Autophagy is activated with persistent ER stress to promote cell survival. ATF4 and CCAAT/enhancer-binding protein (C/EBP) and homologous protein (CHOP) (both activated by PERK) are mostly related with cellular death pathways upon overexpression. Autophagy is transcriptionally regulated by ATF4 and CHOP and can oppose terminal UPR and ATF4 [26]. CHOP has also been identified in the regulation of numerous Atg genes [27]. ER stress also activates c-Jun NH2-terminal kinase (JNK) *via* the interaction of IRE1α and TNF receptor-associated factor 2 (TRAF2), ultimately phosphorylating Bcl-2 and leading to dissociation of Bcl-2 and Beclin-1 proteins. This enables activation of the PI3K complex and initiates autophagy. ER stress-induced activation of apoptosis requires IRE1α, which forms a complex with apoptosis signal-regulating kinase 1 (ASK1) and stimulates its downstream target, JNK, *via* binding to TRAF2. In addition, ER stress induces apoptosis *via* a caspase-dependent pathway. It leads to the translocation of the BH3-only protein, Bcl-2-like protein 11 (BIM), to the ER, leading to caspase-12 activation. Caspase-12 activates caspase-9, leading to downstream caspase-3 activation in this cascade, finally triggering apoptosis. Additionally, IRE1α can cause phosphorylation and activation of CHOP through binding and activation of p38 mitogen-activated protein kinase. CHOP downregulates Bcl-2 and upregulates the transcription of BIM, eventually causing the downstream initiation of an apoptotic cascade [28].

The crosstalk of autophagy with ER stress and apoptosis is illustrated in figure 2.

Recent reports highlight the importance of interplay between ER stress/UPR, autophagy and apoptosis in lung diseases. For example, mevalonate cascade inhibition, which has been linked with improved lung health, leads to cell death *via* coordinated apoptosis, autophagy and ER stress [29]. The interplay between these pathways appears to be mainly regulated *via* autophagy and Bcl-2-family pro-apoptotic proteins [30]. In COPD, the chaperone and master regulator of the UPR-BiP may be upregulated in bronchoalveolar lavage fluid and lung samples taken from smokers. The expression levels of phospho-translation initiation factor 2α (eIF2α) and CHOP correlate with the severity of airway obstruction, and these increments are associated with stress-induced increases in caspase-3 and -7. COPD patients also show a significant elevation in autophagic proteins. Activation of the PERK-eIF2α axis is critical for the autophagy activity associated with ER stress. Further, PERK activates autophagy by inhibiting Akt/ATF4-mediated induction of Atg [28]. The upregulation of autophagy is linked with airway remodelling in asthma [31], as autophagy facilitates the extracellular matrix deposition and fibrosis in asthmatic airway remodelling [30]. ER stress and UPR activation in airway epithelial cells (AECs) adversely affects asthma, and acute and prolonged activation of UPR proteins can lead to the development of an allergic airway inflammation. UPR simultaneously mediates apoptosis in neutrophil cells *via* the activation of the CHOP-PERK arm [25]. A recent study showed that the expression of apoptosis, autophagy and UPR markers correlates with lung function deficits in idiopathic pulmonary fibrosis (IPF). The cell stress markers of BiP, X-box binding

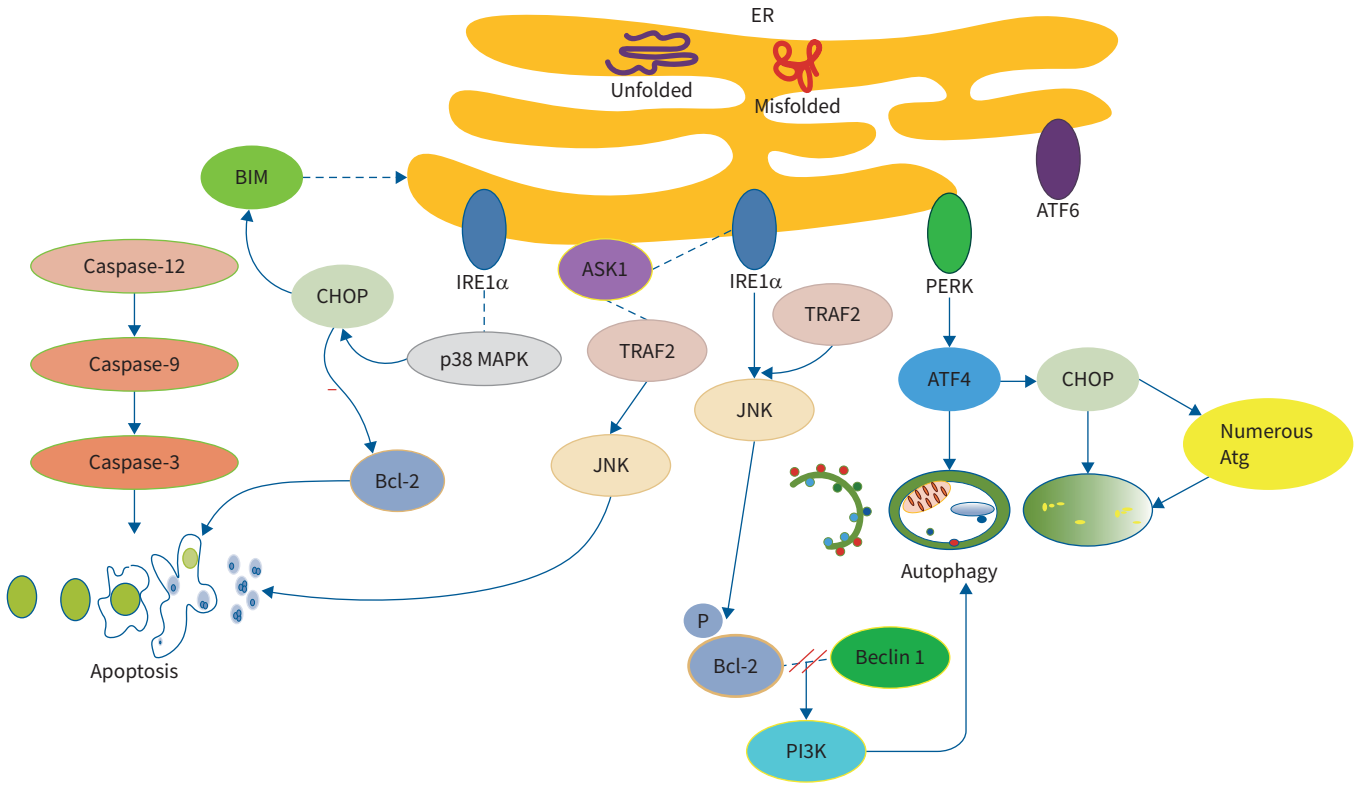

FIGURE 2 The crosstalk of autophagy with endoplasmic reticulum (ER) stress and apoptosis. ER stress activates autophagy to promote cell survival. Autophagy is transcriptionally regulated by acting transcription factor 4 (ATF4) and CCAAT/enhancer-binding protein homologous protein (CHOP) and can oppose terminal unfolded protein response and ATF4. CHOP regulates numerous Atg. ER stress activates c-Jun NH2-terminal kinase (JNK) *via* the interaction of inositol-requiring enzyme 1α (IRE1α) and TNF receptor-associated factor 2 (TRAF2), ultimately phosphorylating Bcl-2 and leading to dissociation of Bcl-2 and Beclin-1 proteins. This enables the activation of the PI3K complex and initiates autophagy. ER-stress-induced activation of apoptosis requires IRE1α. IRE1α forms a complex with apoptosis signal-regulating kinase 1 (ASK1) and stimulates its downstream target, JNK, *via* binding to TRAF2. ER-stress-induced apoptosis *via* a caspase-dependent pathway. ER stress leads to the translocation of Bcl-2-like protein 11 (BIM) to the ER, leading to caspase-12 activation. Caspase-12 activates caspase-9, leading to downstream caspase-3 activation in this cascade, finally triggering apoptosis. Additionally, IRE1α causes the phosphorylation and activation of CHOP through binding and activation of p38 mitogen-activated protein kinase (MAPK). CHOP downregulates Bcl-2 and upregulates the transcription of BIM, eventually causing the downstream initiation of an apoptotic cascade. PERK: protein kinase RNA-like ER kinase.

protein (XBP1), LC3β puncta and cleaved caspase-3 were elevated in IPF lungs compared to non-IPF lungs. However, the mechanisms linking UPR and autophagy in IPF and the imbalance in these cell stress pathways requires further research [24].

Roles of autophagy in ageing-related pulmonary diseases

Acute lung injury (ALI)

The incidence and mortality of ALI and severe ARDS markedly increase with advancing age [32]. ALI is a common and severe clinical syndrome, characterised by noncardiogenic pulmonary oedema, increased alveolar-capillary permeability, neutrophil recruitment and diffused alveolar damage, and is a major cause of acute respiratory failure [33]. The inflammatory response in the lungs with the release of proinflammatory cytokines can be observed in the pathogenesis of ALI [34, 35]. The involved wound repair mechanism, namely, the fibro-proliferative response, if excessive and persistent, will lead to interstitial fibrosis [36, 37].

Evidence proves that autophagy is stimulated in response to various stimuli of ALI, such as bacterial infection, lipopolysaccharide, sepsis, hyperoxia and COVID-19 [1, 38, 39]. The loss of Atg genes, such as Atg7, Atg5 and Atg4B, markedly aggravates the development of ALI in mice [38, 40], indicating that autophagy has protective effects against the initiation and progression of ALI in certain contexts.

COPD

COPD is the fourth leading cause of death worldwide, with increasing prevalence, particularly in the elderly, and ageing hallmarks are prominent features of COPD. COPD is characterised by dysfunctional tissue repair, resulting in (small) airway diseases and emphysema, manifested as persistent respiratory symptoms and airflow limitations [41]. Cigarette smoke extract (CSE) exposure is the most common risk factor for COPD [42, 43]. The pathogenesis of COPD remains incompletely understood; however, increasing evidence has shown that autophagy may be involved in the pathogenesis of COPD. *In vitro* studies using lower doses of CSE have shown that loss of autophagy enhances smoke-induced epithelial cell senescence, mitochondrial reactive oxygen species (ROS) production, and the accumulation of ubiquitinated proteins along with the accumulation of sequestosome 1 [44–46], suggesting that autophagy plays a protective role in epithelial cells in the pathogenesis of COPD. The chemical activation of autophagy also protects cells *in vitro* [45] and *in vivo* from smoke exposure [47]. However, other studies conversely demonstrated that the activation of autophagy, particularly selective autophagy, has harmful effects on epithelial cells in response to smoke [48–51]. CSE increases autophagosomal turnover (flux) and promotes epithelial cell death both *in vitro* and *in vivo* [52, 53] and then initiates and exaggerates airway inflammation [54] and mucus hyperproduction [55]. These contradictory results suggest the dual role of autophagy in COPD.

Asthma

Asthma is a complex disorder of the airways involving bronchial hyperreactivity, chronic airway inflammation, mucus overproduction and airway wall remodelling [56, 57]. Ageing-associated change in immune responses facilitates the pathogenesis of asthma in the elderly [58]. Autophagy is mainly involved in the pathogenesis of asthma *via* the regulation of the body's innate and adaptive immune responses [59]. It is involved in several key processes of asthma pathogenesis, such as airway hyperresponsiveness [60], eosinophilic airway inflammation [61] and airway remodelling [31]. It may also promote interleukin (IL)-18 secretion in response to outdoor allergens in AECs [62]. Bronchial fibroblasts in patients with severe asthma exhibit increased mitophagy and expression of PTEN-inducible putative kinase 1 (PINK1) and Parkin, as well as an increased light chain 3 phosphatidylethanolamine conjugate (LC3-II) expression and a profibrotic phenotype [63]. *In vitro* human AECs showed that IL-13 stimulates goblet cell formation and mucin 5AC secretion, correlating with the activation of autophagy, manifested by an increase in LC3-II and increased autophagic flux, which can be prevented by *Atg5* knockdown [64]. Similarly, Atg5 is correlated with reduced lung function and airway remodelling in patients with severe asthma [65].

IPF

IPF is an interstitial lung disease characterised by massive deposition of the extracellular matrix in the lung interstitium and the irreversible and slowly progressive destruction of lung structure and function [66]. The aetiology of IPF is unknown and ageing is one of the most significant risk factors for IPF. Fibroblast senescence establishes a close link between cellular senescence and IPF [67], senolytics that remove senescent fibroblasts decreased pulmonary fibrosis (PF) in a mouse model of IPF [68, 69].

The induction or enhancement of autophagy has anti-fibrosis effects; autophagy deficiency can promote the deposition of extracellular matrix in lung fibroblasts and accelerate the process of fibrosis [70, 71]. Reduced autophagy can induce epithelial–mesenchymal transition of AECs and contribute to fibrosis *via* aberrant epithelial–fibroblast crosstalk [72]. However, another study showed that autophagy may also promote the profibrotic effects of transforming growth factor β1 in human lung fibroblasts [73]. Moreover, Akt1-mediated mitophagy contributes to alveolar macrophage apoptosis resistance and PF development [74]. Further studies addressing the regulatory network of autophagy in PF are necessary.

The specific autophagy receptors and markers that change in lung diseases are summarised in table 1.

The anti-ageing mechanism of autophagy in pulmonary dysfunction

Ageing results in physiological, structural and mechanical changes that diminish lung function. In this condition, insults to the ageing lung are more likely to lead to pathological repair rather than wound resolution and restitution in the lungs. Animal studies proved that older mice are more prone to lung injury; older mice take a longer time to recover than younger mice [75, 76]. Increasing evidence proves that the activity of autophagy is altered in the ageing lung and modulating autophagy may be a promising strategy for treating lung diseases, particularly those in the elderly.

Autophagy maintains protein homeostasis

Loss of proteostasis is a hallmark of ageing [8]. Advanced age itself alone can impair the physiological function of the lung even in the absence of diseases [2]. When the lung faces various biochemical

TABLE 1 The specific autophagy receptors and markers that change in lung diseases

Disease	Changes in autophagy receptors and markers	Reference
ALI	Atg7, Atg5, Atg4B	[41, 49]
COPD	PINK1, PARK2, p62, LC3-II, Beclin-1, histone deacetylase 6, Bcl-2:XIAP, PARP, CD63, LAMP1	[56–58, 64–66]
Asthma	LC3, Atg5, Beclin-1, p62, Zn, ZIP1, ZIP2, PINK1, Parkin	[37, 73–77]
IPF	PI3K/Akt pathway, PTEN, Beclin-1:mTOR signalling pathway, Atg7, Bcl-2 complex, p62, LC3-II, LC3βII, Atg5–12	[86–88]

ALI: acute lung injury; IPF: idiopathic pulmonary fibrosis; LAMP1: lysosomal-associated membrane protein 1; LC3: light chain 3; LC3-II: light chain 3 phosphatidylethanolamine conjugate; mTOR: mammalian target of rapamycin; PARP: poly-(ADP)-ribose polymerase; PINK1: PTEN-inducible putative kinase 1; PTEN: phosphatase and tensin homologue; XIAP: X-linked inhibitor of apoptosis protein; ZIP: zinc transporter.

perturbations over a lifetime, it leads to impaired protein homeostasis that urge a robust response to resultant proteotoxic stress. Proteomic analysis of type II AECs isolated from young and old mice revealed a maladaptive collapse of the proteostasis network due to age and the critical role for the co-chaperone adaptive response network in handling chronic misfolded proteins in the ageing healthy lung [2]. Several genetic respiratory diseases are attributable to protein misfolding due to underlying genetic mutations. For example, mutations in the surfactant protein C gene and in the mucin 5B promoter region [77, 78] are related to PF.

Along with the ubiquitin–proteasome system, autophagy is a principal proteolytic system that plays a central role in maintaining cellular proteostasis [8]. The genetic inhibition of core components or regulators of the autophagy machinery markedly accelerates age-related protein aggregation, manifested as shortened lifespan and exacerbated pathological features in worm, fly and animal models of diseases. Disturbance of the interrelationship between proteasomes and autophagy pathways may lead to the accumulation of aberrant proteins and eventually result in pathological conditions in the lungs. For example, smoke-induced aggresome formation contributes to the COPD–emphysema pathogenesis and is related to impaired autophagy; autophagy-inducing drugs markedly decrease aggresome colocalisation and expression [47, 79]. In acute injury, such as chemical lung injury with bleomycin, autophagy decreases with the corresponding elevated levels of oxidised proteins and lipofuscin in response to lung injury in old and middle-aged mice compared with that in younger animals. Older mice with lung injury are characterised by deficient autophagic response and reduced mitophagy [80].

Autophagy modulates stem cell function

Reduction in the regenerative potential of tissues is one of the most obvious characteristics of ageing [8]. In the airways, basal cells (BCs) are multipotent stem cells that form part of the pseudostratified epithelium of the bronchi and trachea. Type II AECs are the main progenitor cell population in the alveolar parenchyma. Type II AECs regenerate after injury by proliferating and differentiating into type I cells, which are critical to gas exchange [81]. Age can cause both quantitative and qualitative changes in various progenitor populations in the lungs. For example, BCs and club cells decrease in number with age, whereas type II AECs do not decrease in quantity but exhibit reductions in self-renewal and differentiation capacity [82–84.]

Dysregulation of stem cell maintenance has been implicated in the pathogenesis of chronic lung diseases; for example, chronic exposure to cigarette smoke (CS) induces a proinflammatory microenvironment that distorts the BC transcriptome, generating pathological epithelial phenotypes [85]. The eventual progenitor exhaustion is responsible for the pathogenesis of COPD [86]. The mechanism through which ageing reshapes the lung stem niche still needs to be elucidated and declined autophagy may be linked to stem cell function reduction during the ageing process. CS-induced senescence in type II AECs is related to decreased autophagy, which enhances p21-mediated cellular senescence [87] and promotes apoptosis in type II AECs [88], while both apoptosis and senescence in type II AECs contribute to the emphysema observed in patients with COPD. Questions remain about how autophagy alters the contributions of epithelial, mesenchymal and other cell populations to lung regeneration during the ageing process.

Autophagy maintains genomic stability

The accumulation of genetic damage throughout life is a common denominator of ageing and genomic instability is a primary hallmark of ageing [8]. DNA damage may affect essential genes and

transcriptional pathways, cause subevent dysfunction in cells and, if not eliminated, may jeopardise homeostasis in cells.

DNA damage is prominent in the lung cells of patients with COPD under a hypoxic response condition [89]. Increased breaks in DNA double strands and DNA repair inefficiency may be associated with increased levels of oxidative stress; these are correlated with an increased susceptibility to the development and progression of diseases [90]. Furthermore, mitochondrial DNA (mtDNA) damage and apoptosis of AECs mediated by Sirtuin (SIRT) 3 deficiency promote PF [91]. Genome instability also amplified by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The coronavirus papain-like protease degrades transcription factor p53 and allows the replication of infected cells [92], while, in addition to participating in the DNA damage response (DDR) and contributing to the ageing process [93], p53 can downregulate coronavirus replication by regulating the cell cycle [94].

The DDR is an evolutionary conserved signalling network responsible for DNA lesion sensing and DNA repair. DDR-induced autophagy selectively degrades distinct proteins, such as ribonucleotide reductase, ubiquitin-specific protease 14 and checkpoint kinase 1, which directly or indirectly influence DNA repair and cell fate, such as proliferation, apoptosis and senescence [95–97]. DNA can be directly degraded through autophagy. There also exists nucleophagy, which is conserved in mammalian cells [98] and may be a defence mechanism that protects cells from tumorigenesis.

Autophagy has a protective effect against DNA damage in ageing-related lung diseases. For example, CS is a strong risk factor for IPF and is also a pro-senescent factor. Zhang *et al.* [99] found that CSE reduced autophagy and mitophagy and increased mitochondrial ROS in murine lung epithelial-12 cells, and that CSE promoted DNA damage, downregulated $tNAD^+$/NADH ratio and suppressed SIRT1 activity. Autophagy inducer rapamycin and the mitochondria-targeted antioxidant mitoquinone inhibited CSE-related senescence and decreased mitochondrial ROS and that activating SIRT1 attenuated senescence through an autophagy-dependent pathway [99]. In sepsis-induced ALI, the level of circulating mtDNA and the degree of STING (stimulator of interferon genes) activation are increased, mtDNA evoked an inflammatory storm and disturbed autophagy, inducing autophagy or STING deficiency can markedly alleviate lung injury [100]. These data suggest that autophagic modulation of DNA haemostasis is essential for health improvement, ageing and the prevention of diseases in the lungs.

Mitophagy regulates mitochondrial quality control

Mitochondria are “powerhouses” that produce cellular energy in the form of ATP. As cells and organisms age, the efficacy of the electron transport chain (ETC) tends to reduce, accompanied by increased electron leakage and reduced ATP generation [101]. Mitochondrial dysfunction contributes to physical ageing and a wide spectrum of age-related diseases, particularly lung diseases [102]. Mitochondrial dysfunction may increase the risk of severe COVID-19 outcomes; in those who have COVID-19, the increased energy expenditure secondary to a cytokine storm can lead to a nonadaptive state, overwhelming the metabolic reserve capacity of the mitochondria [103].

One study showed that the activation of mitophagy is required to extend the lifespan in several long-lived *C. elegans* mutants, including the insulin-like growth factor (IGF)/IGF-1 mutants [104]. In the lungs, mitophagy plays an important role in the pathogenesis of ageing-associated pulmonary disorders. The knockdown of both PINK1 and PARK2 enhances the senescence of human bronchial epithelial cells (HBECs) in response to CSE and is accompanied by accumulated damage to the mitochondria and increased ROS production [44]. Moreover, impaired mitophagy also participates in the regulation of myofibroblast differentiation in lung fibroblasts [87, 105]. Insufficient mitophagy due to PARK2 deficiency induces mtROS production, accompanied by activated platelet-derived growth factor receptor/mammalian target of rapamycin (mTOR) signalling, which causes myofibroblast differentiation and proliferation [105].

Autophagy regulates cellular senescence

Increased cellular senescence is a hallmark of ageing [8]. As the number of senescent cells increases with age, senescence may be a beneficial compensatory response to rid tissues of damaged and potentially oncogenic cells. However, this cellular checkpoint also requires an efficient cell replacement system that contributes to the clearance of senescent cells and mobilisation of progenitors to re-establish cell numbers [8]. Senescent cells are apoptosis-resistant and secrete numerous factors termed the “senescence-associated secretory phenotype” (SASP) [106, 107]. The SASP mediates many of their pathophysiological effects and contributes to age-related conditions [108].

In the lungs, mouse studies showed the causal relation of senescence to lung ageing and diseases; senescent-prone recombinant mice exhibit accelerated lung ageing and are more susceptible to experimental lung injury [109, 110]. In contrast, the ablation of senescence cells in the lung *in vivo* prolongs life and has a restorative effect on lung tissue [111, 112]. In IPF, lung fibrosis is mediated, in part, by senescent cells; cellular senescence markers are detectable within lung tissue and senescent cell deletion rejuvenates pulmonary health in old mice [69]. Furthermore, cellular senescence has been implicated in the pathogenesis of COPD and asthma [113, 114]. Recently, Lee *et al*. [115] found that virus-induced senescence is also a pathogenic trigger of COVID-19-related cytokine escalation and organ damage, and senolytic targeting of virus-infected cells can be a treatment option against SARS-CoV-2 and other viral infections.

The detailed molecular mechanism of the regulation of cellular senescence is complex and yet to be clarified; it has been proven that autophagy insufficiency plays a pivotal role in the accumulation of deleterious cellular components—as one of the typical manifestations of cellular senescence is the accumulation of damaged proteins and organelles, occasionally associated with ubiquitinated aggregations. Cellular senescence induces autophagy through the inhibition of mTOR and the activation of AMPK and SIRT1 [116, 117]. SIRT1 deacetylation of Atg proteins and transcription factors is involved in autophagy induction [118].

In lung diseases, such as CS-induced COPD, CSE transiently induces autophagy activation with subsequent accumulation of sequestosome-1 and ubiquitinated proteins and an increase in HBEC senescence. The inhibition of autophagy further enhances HBEC senescence, accompanied by the accumulation of sequestosome-1 and ubiquitinated proteins, which reflect insufficient autophagic degradation; moreover, the knockdown of both PINK1 and PARK2 enhances HBEC senescence in response to CSE, accompanied by the accumulation of damaged mitochondria and increased ROS production [44]. However, other studies showed that autophagy may facilitate cellular senescence and that autophagy and senescence may occur in parallel. For example, the overexpression of the Atg gene Unc-51 like autophagy activating kinase (ULK) 3 can induce both autophagy and senescence, whereas the inhibition of autophagy delayed the senescence phenotype, including the SASP [119, 120]. Nonetheless, it can be inferred that autophagy and cellular senescence are interrelated and these two important cellular processes may be dependently and interdependently involved in the pathophysiology of lung diseases.

Autophagy regulates inflammation and inflammageing

Inflammation is an adaptation mechanism designed to maintain organismal homeostasis and inhibit acute and local perturbations, specifically in response to infection or injury [121]. “Inflammageing”, first termed by Franceschi *et al*. [122], describes a chronic, sterile, low-grade inflammation that contributes to the pathogenesis of age-related diseases. In the lungs, inflammation arises due to persistent exposure to various stimuli and stress. For example, the “cytokine storm” frequently observed in patients with ARDS with severe COVID-19 infection is thought to be a consequence of inflammageing [123, 124]. Older individuals are more susceptible to lung injury due to dysregulated response compared with that in younger individuals.

The exact origin and drivers of inflammageing remain yet to be clarified and may involve multiple ageing mechanisms that occur throughout the body (figure 3). Franceschi *et al*. [122] proposed that in the ageing process, the accumulation of “cellular garbage”, represented by endogenous/self, misplaced or altered molecules due to damaged and/or dead cells and organelles (cell debris), acts as damage-associated molecular patterns (DAMPs) that activate an auto-inflammatory response by binding to pattern recognition receptors (PRRs) on innate immune cells, particularly macrophages [122]. Age-related declines in autophagy and macrophage phagocytic activity contribute to DAMP accumulation and lead to inflammation and ageing.

By eliminating the debris and products of cellular metabolism, autophagy promotes the recycling of cellular content, thus generating nutrients and energy to maintain homeostasis and preventing the recognition of DAMPs by PRRs and the consequent inflammation [125]. Furthermore, autophagy could control inflammation through the regulation of innate immunity signalling by removing endogenous inflammasome agonists and through effects on the section of immune mediators. Additionally, autophagy modulates antigen presentation and T-cell homeostasis and affects T-cell repertories and polarisation [126]. The promotion of autophagy through starvation or rapamycin administration inhibits the activation of inflammasome [127].

Autophagy regulates oxidative stress

In biological systems, oxidative stress is generated when endogenous antioxidant defences are impaired and/or overwhelmed by the presence of ROS [128]. Ageing hallmarks contributing to the ageing process

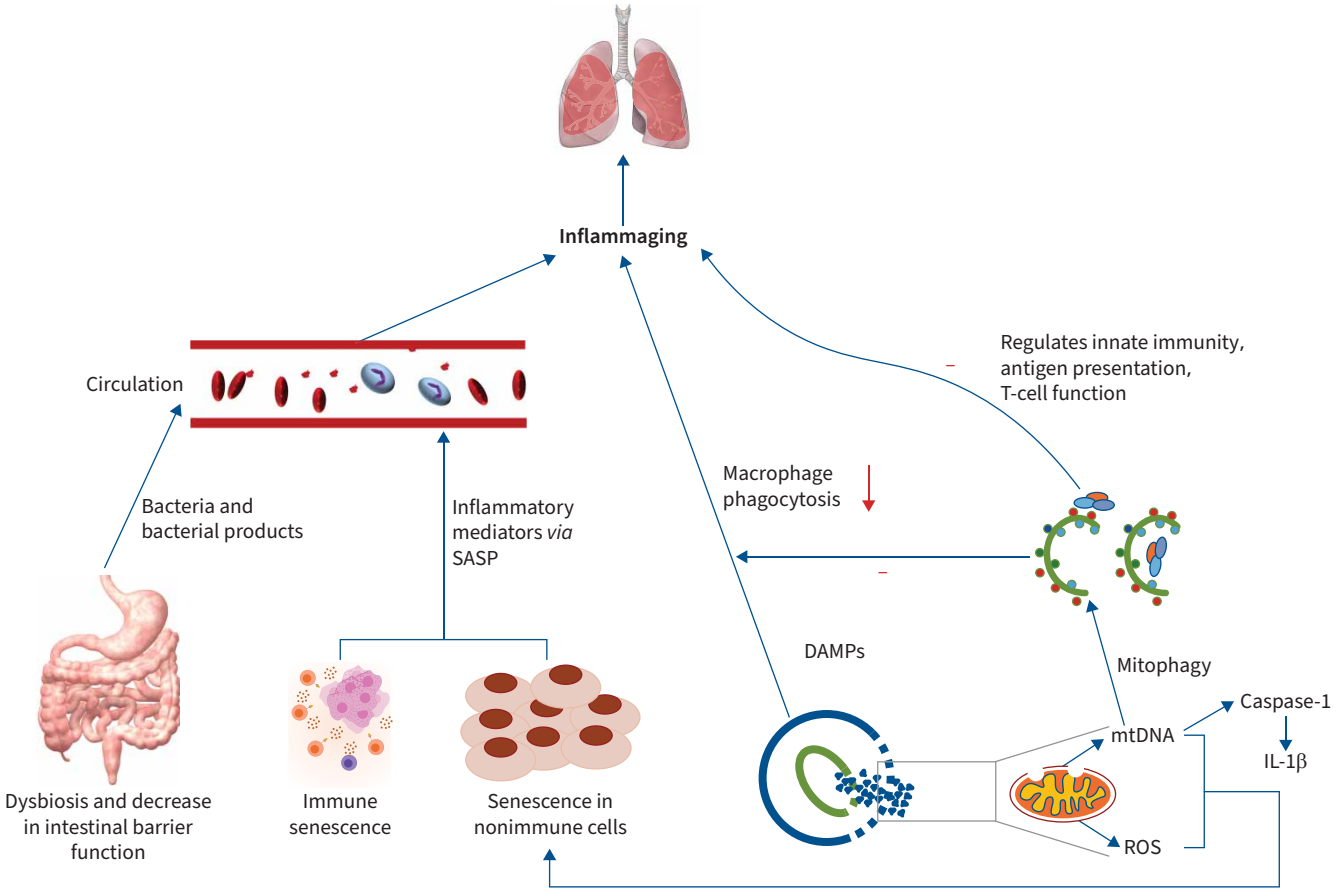

FIGURE 3 Multiple mechanisms contributing to inflammaging and the involvement of autophagy. Multiple mechanisms contribute to inflammaging, eventually leading to lung pathogenesis. Senescence in immune and nonimmune cells increases inflammatory mediator release *via* the release of senescence-associated secretory phenotype (SASP). In aged intestines, dysbiosis of intestinal microbiota and a decreased intestinal barrier allow bacterial products to translocate to the circulatory system, where they trigger low systemic inflammation. Damaged and/or dead cells and organelles act as damage-associated molecular patterns (DAMPs) to activate an auto-inflammatory response by binding to pattern recognition receptors (PRRs) on innate immune cells. Age-related declines in autophagy and macrophage phagocytic activity also contribute to DAMP accumulation and lead to inflammation and ageing. Autophagy promotes recycling cellular content, generating nutrients and energy to maintain homeostasis, preventing recognition of pathogen-associated molecular patterns by PRRs and inflammation. Autophagy can also control inflammation *via* the regulation of innate immunity signalling by removing endogenous inflammasome agonists and through its effects on immune mediator secretion. Additionally, autophagy modulates innate immunity, antigen presentation and T-cell function. Furthermore, mitophagy inhibits mitochondrial DNA (mtDNA) accumulation and excessive mitochondrial reactive oxygen species (ROS) production, both of which may accelerate cellular senescence and aggravate the inflammaging process. IL-1β: interleukin 1 beta.

could also be caused by oxidative stress. For example, telomeres are highly sensitive to oxidative stress and their repair capacity is poor compared with that of other parts of the chromosome [129].

Autophagy is activated in response to oxidative stress to protect the cells from apoptosis [130]. Increased oxidative stress is sensed by the lysosomal cation channel, mucolipin 1 [131]. Mucolipin 1 oxidation promotes channel opening; Ca^{2+} is released from the lysosomal lumen and the Ca^{2+}-dependent phosphatase, calcineurin, is activated [132]. Calcineurin-dependent transcription factor E (TFEB) phosphorylation promotes TFEB translocation to the nucleus and stimulates autophagy [132]. ROS can also activate autophagy by inhibiting the PI3K-Akt or by activating AMPK to inhibit the mTOR signalling pathway [133]. Lung diseases are inevitably accompanied by hypoxia, which is a major stimulus for the induction of autophagy [134]. Hypoxia can impair mitochondrial ETC activity, increase mitochondrial $O_2^{\cdot-}$ production and result in mitochondrial dysfunction [134, 135]. Chronic and moderate hypoxia trigger hypoxia-inducible factor-1α and delta isoform of protein kinase C–JNK1-mediated pathways to activate autophagy [136]. Oxidative stress has been identified as the major contributor to the dysregulated response and intractable inflammation under CSE [137]. CSE causes mitochondrial damage and accumulation by

impairing mitophagy in a deteriorative state; mitophagy protects HBECs by removing damaged mitochondria and reducing the production of ROS [44].

Collectively, autophagy represents a negative feedback mechanism of regulation, which eliminates the accumulation of ROS and protects cells from oxidative injury; this is particularly critical in ageing-related lung diseases.

Autophagy regulates immune function

The immune system cannot escape from the effects of ageing and displays senescence characteristics in aged individuals, that is, immunosenescence, producing a chronic inflammatory status of the organism, as manifested by a decreased ability to counteract antigens [138].

Immunosenescence and a corresponding decrease in immune regulation are implicated in age-related lung diseases. Elderly individuals face a greater risk of many diseases, particularly respiratory diseases, due to their poor response to immune challenges compared with younger ones.

Autophagic activity decreases with age in immune cells. Decreased activities of autophagy and mitophagy in phagocytic cells may impair their ability to kill pathogens and arouse dysregulated activation of inflammasomes, which increases the production of inflammatory cytokines and contributes to the age-related inflammageing phenotype.

The human respiratory tract is an important immune interface requiring a tightly regulated response to the continuous exposure to environmental stress [2]. Manoeuvring autophagy to tune immune function and immunosenescence can be a promising therapeutic management in ageing and ageing-related lung diseases.

Conclusions

Autophagy plays a central role in the regulation of ageing. Pulmonary diseases are characterised by a dysregulation of autophagic activity, particularly in those at an advanced age in several species. The detailed mechanism of autophagy in physiological ageing and pathological conditions still need to be further explored.

Enhancing autophagy generally promotes cellular functions and homeostasis, thereby prolonging lifespan and improving pulmonary health. However, it should be noted that significantly increasing autophagy may shorten the lifespan and harm the lungs [23]. Targeting autophagy for therapeutic exploitation in ageing and ageing-related lung diseases depends on the exact nature of the autophagic defect present in each cell type. In the studies discussed in this review, the exact autophagic defects contributing to ageing-related lung diseases are yet to be fully elucidated. This can be partly attributed to the limited specificity of current autophagy modulators in targeting one cell type in lung tissue.

Based on current literature, it can be hypothesised that the possibility of improving autophagy activity to boost cell function in lungs offers a very attractive therapeutic approach to enhance lung function in the elderly. For example, modulating autophagy through calorie restriction, exercise can be a promising therapeutic agent for various pulmonary diseases [139, 140]. Long-term health benefits for the lungs will likely arise from achieving the right balance of autophagy, which will depend on lung tissue and organismal age. Furthermore, the use of autophagy modulators (inductors/inhibitors) combined with other diverse therapies provides a promising strategy to treat a variety of pulmonary conditions.

Provenance: Submitted article, peer reviewed.

Published in volume 31, issue 166 of the European Respiratory Review on 21 December 2022; republished 5 January 2023 with amendments to the authors' affiliation details.

Author contributions: Z. Fu formulated the idea and collected the literature, Y. Zhang organised the paper and created the figures, and Z. Fu wrote the manuscript. J. Zhang reviewed the manuscript. All authors read and approved the final manuscript.

Conflicts of interest: The authors declare no conflict of interest.

References

1 Wang K, Chen Y, Zhang P, *et al.* Protective features of autophagy in pulmonary infection and inflammatory diseases. *Cells* 2019; 8: 123.

2 Schneider JL, Rowe JH, Garcia-de-Alba C, *et al.* The aging lung: physiology, disease, and immunity. *Cell* 2021; 184: 1990–2019.
3 Campisi J, Kapahi P, Lithgow GJ, *et al.* From discoveries in ageing research to therapeutics for healthy ageing. *Nature* 2019; 571: 183–192.
4 Partridge L, Deelen J, Slagboom PE. Facing up to the global challenges of ageing. *Nature* 2018; 561: 45–56.
5 Fang EF, Xie C, Schenkel JA, *et al.* A research agenda for ageing in China in the 21st century (2nd edition): focusing on basic and translational research, long-term care, policy and social networks. *Ageing Res Rev* 2020; 64: 101174.
6 Spagnolo P, Balestro E, Aliberti S, *et al.* Pulmonary fibrosis secondary to COVID-19: a call to arms? *Lancet Respir Med* 2020; 8: 750–752.
7 Koff WC, Williams MA. Covid-19 and immunity in aging populations - a new research agenda. *N Engl J Med* 2020; 383: 804–805.
8 López-Otín C, Blasco MA, Partridge L, *et al.* The hallmarks of aging. *Cell* 2013; 153: 1194–1217.
9 Shin CH, Kim KH, Jeeva S, *et al.* Towards goals to refine prophylactic and therapeutic strategies against COVID-19 linked to aging and metabolic syndrome. *Cells* 2021; 10: 1412.
10 Cho SJ, Stout-Delgado HW. Aging and lung disease. *Annu Rev Physiol* 2020; 82: 433–459.
11 Murtha LA, Morten M, Schuliga MJ, *et al.* The role of pathological aging in cardiac and pulmonary fibrosis. *Aging Dis* 2019; 10: 419–428.
12 Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell* 2019; 176: 11–42.
13 Pinto C, Ninfole E, Benedetti A, *et al.* Involvement of autophagy in ageing and chronic cholestatic diseases. *Cells* 2021; 10: 2772.
14 Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 2011; 27: 107–132.
15 Klionsky DJ, Petroni G, Amaravadi RK, *et al.* Autophagy in major human diseases. *EMBO J* 2021; 40: e108863.
16 Yeganeh B, Lee J, Ermini L, *et al.* Autophagy is required for lung development and morphogenesis. *J Clin Invest* 2019; 129: 2904–2919.
17 Chang JT, Kumsta C, Hellman AB, *et al.* Spatiotemporal regulation of autophagy during Caenorhabditis elegans aging. *eLife* 2017; 6: e18459.
18 Simonsen A, Cumming RC, Brech A, *et al.* Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult Drosophila. *Autophagy* 2008; 4: 176–184.
19 Denzel MS, Lapierre LR, Mack HID. Emerging topics in *C. elegans* aging research: transcriptional regulation, stress response and epigenetics. *Mech Ageing Dev* 2019; 177: 4–21.
20 Lapierre LR, Kumsta C, Sandri M, *et al.* Transcriptional and epigenetic regulation of autophagy in aging. *Autophagy* 2015; 11: 867–880.
21 Tóth ML, Sigmond T, Borsos E, *et al.* Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. *Autophagy* 2008; 4: 330–338.
22 Pyo JO, Yoo SM, Ahn HH, *et al.* Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat Commun* 2013; 4: 2300.
23 Bjedov I, Cochemé HM, Foley A, *et al.* Fine-tuning autophagy maximises lifespan and is associated with changes in mitochondrial gene expression in Drosophila. *PLoS Genet* 2020; 16: e1009083.
24 Sharma P, Alizadeh J, Juarez M, *et al.* Autophagy, apoptosis, the unfolded protein response, and lung function in idiopathic pulmonary fibrosis. *Cells* 2021; 10: 1642.
25 Dastghaib S, Kumar PS, Aftabi S, *et al.* Mechanisms targeting the unfolded protein response in asthma. *Am J Respir Cell Mol Biol* 2021; 64: 29–38.
26 Sano R, Reed JC. ER stress-induced cell death mechanisms. *Biochim Biophys Acta* 2013; 1833: 3460–3470.
27 B'Chir W, Maurin AC, Carraro V, *et al.* The eIF2α/ATF4 pathway is essential for stress-induced autophagy gene expression. *Nucleic Acids Res* 2013; 41: 7683–7699.
28 Aghaei M, Dastghaib S, Aftabi S, *et al.* The ER stress/UPR axis in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Life* 2020; 11: 1.
29 Ghavami S, Sharma P, Yeganeh B, *et al.* Airway mesenchymal cell death by mevalonate cascade inhibition: integration of autophagy, unfolded protein response and apoptosis focusing on Bcl2 family proteins. *Biochim Biophys Acta* 2014; 1843: 1259–1271.
30 Zeki AA, Yeganeh B, Kenyon NJ, *et al.* Autophagy in airway diseases: a new frontier in human asthma? *Allergy* 2016; 71: 5–14.
31 McAlinden KD, Deshpande DA, Ghavami S, *et al.* Autophagy activation in asthma airways remodeling. *Am J Respir Cell Mol Biol* 2019; 60: 541–553.
32 Rubenfeld GD, Caldwell E, Peabody E, *et al.* Incidence and outcomes of acute lung injury. *N Engl J Med* 2005; 353: 1685–1693.
33 Bhattacharya J, Matthay MA. Regulation and repair of the alveolar-capillary barrier in acute lung injury. *Annu Rev Physiol* 2013; 75: 593–615.

34 Matute-Bello G, Frevert CW, Martin TR. Animal models of acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L379–L399.
35 Lang JD, McArdle PJ, O'Reilly PJ, *et al.* Oxidant-antioxidant balance in acute lung injury. *Chest* 2002; 122: 314s–320s.
36 Frenzel J, Gessner C, Sandvoss T, *et al.* Outcome prediction in pneumonia induced ALI/ARDS by clinical features and peptide patterns of BALF determined by mass spectrometry. *PLoS One* 2011; 6: e25544.
37 Cheng P, Li S, Chen H. Macrophages in lung injury, repair, and fibrosis. *Cells* 2021; 10: 436.
38 Li ZY, Wu YF, Xu XC, *et al.* Autophagy as a double-edged sword in pulmonary epithelial injury: a review and perspective. *Am J Physiol Lung Cell Mol Physiol* 2017; 313: L207–L217.
39 Bello-Perez M, Sola I, Novoa B, *et al.* Canonical and noncanonical autophagy as potential targets for COVID-19. *Cells* 2020; 9: 1619.
40 Aggarwal S, Mannam P, Zhang J. Differential regulation of autophagy and mitophagy in pulmonary diseases. *Am J Physiol Lung Cell Mol Physiol* 2016; 311: L433–L452.
41 Rodriguez-Roisin R, Rabe KF, Vestbo J, *et al.* Global initiative for chronic obstructive lung disease (GOLD) 20th anniversary: a brief history of time. *Eur Respir J* 2017; 50: 1700671.
42 Rabe KF, Hurd S, Anzueto A, *et al.* Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007; 176: 532–555.
43 Strzelak A, Ratajczak A, Adamiec A, *et al.* Tobacco smoke induces and alters immune responses in the lung triggering inflammation, allergy, asthma and other lung diseases: a mechanistic review. *Int J Environ Res Public Health* 2018; 15: 1033.
44 Ito S, Araya J, Kurita Y, *et al.* PARK2-mediated mitophagy is involved in regulation of HBEC senescence in COPD pathogenesis. *Autophagy* 2015; 11: 547–559.
45 Fujii S, Hara H, Araya J, *et al.* Insufficient autophagy promotes bronchial epithelial cell senescence in chronic obstructive pulmonary disease. *Oncoimmunology* 2012; 1: 630–641.
46 Roscioli E, Tran HB, Jersmann H, *et al.* The uncoupling of autophagy and zinc homeostasis in airway epithelial cells as a fundamental contributor to COPD. *Am J Physiol Lung Cell Mol Physiol* 2017; 313: L453–L465.
47 Tran I, Ji C, Ni I, *et al.* Role of cigarette smoke-induced aggresome formation in chronic obstructive pulmonary disease-emphysema pathogenesis. *Am J Respir Cell Mol Biol* 2015; 53: 159–173.
48 Lam HC, Cloonan SM, Bhashyam AR, *et al.* Histone deacetylase 6-mediated selective autophagy regulates COPD-associated cilia dysfunction. *J Clin Invest* 2013; 123: 5212–5230.
49 An CH, Wang XM, Lam HC, *et al.* TLR4 deficiency promotes autophagy during cigarette smoke-induced pulmonary emphysema. *Am J Physiol Lung Cell Mol Physiol* 2012; 303: L748–L757.
50 Chen ZH, Kim HP, Sciurba FC, *et al.* Egr-1 regulates autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. *PLoS One* 2008; 3: e3316.
51 Mizumura K, Cloonan SM, Nakahira K, *et al.* Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. *J Clin Invest* 2014; 124: 3987–4003.
52 Wang G, Zhou H, Strulovici-Barel Y, *et al.* Role of OSGIN1 in mediating smoking-induced autophagy in the human airway epithelium. *Autophagy* 2017; 13: 1205–1220.
53 Hou HH, Cheng SL, Chung KP, *et al.* Elastase induces lung epithelial cell autophagy through placental growth factor: a new insight of emphysema pathogenesis. *Autophagy* 2014; 10: 1509–1521.
54 Li D, Hu J, Wang T, *et al.* Silymarin attenuates cigarette smoke extract-induced inflammation via simultaneous inhibition of autophagy and ERK/p38 MAPK pathway in human bronchial epithelial cells. *Sci Rep* 2016; 6: 37751.
55 Zhou JS, Zhao Y, Zhou HB, *et al.* Autophagy plays an essential role in cigarette smoke-induced expression of MUC5AC in airway epithelium. *Am J Physiol Lung Cell Mol Physiol* 2016; 310: L1042–L1052.
56 Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med* 2012; 18: 684–692.
57 Papi A, Brightling C, Pedersen SE, *et al.* Asthma. *Lancet* 2018; 391: 783–800.
58 Busse PJ, Mathur SK. Age-related changes in immune function: effect on airway inflammation. *J Allergy Clin Immunol* 2010; 126: 690–9; quiz 700-1.
59 Lv X, Li K, Hu Z. Asthma and autophagy. *Adv Exp Med Biol* 2020; 1207: 581–584.
60 Liu JN, Suh DH, Trinh HK, *et al.* The role of autophagy in allergic inflammation: a new target for severe asthma. *Exp Mol Med* 2016; 48: e243.
61 Lee J, Kim HS. The role of autophagy in eosinophilic airway inflammation. *Immune Netw* 2019; 19: e5.
62 Murai H, Okazaki S, Hayashi H, *et al.* Alternaria extract activates autophagy that induces IL-18 release from airway epithelial cells. *Biochem Biophys Res Commun* 2015; 464: 969–974.
63 Ramakrishnan RK, Bajbouj K, Hachim MY, *et al.* Enhanced mitophagy in bronchial fibroblasts from severe asthmatic patients. *PLoS One* 2020; 15: e0242695.
64 Dickinson JD, Alevy Y, Malvin NP, *et al.* IL13 activates autophagy to regulate secretion in airway epithelial cells. *Autophagy* 2016; 12: 397–409.

65 Martin LJ, Gupta J, Jyothula SS, *et al.* Functional variant in the autophagy-related 5 gene promotor is associated with childhood asthma. *PLoS One* 2012; 7: e33454.
66 Raghu G, Collard HR, Egan JJ, *et al.* An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011; 183: 788–824.
67 Blokland KEC, Waters DW, Schuliga M, *et al.* Senescence of IPF lung fibroblasts disrupt alveolar epithelial cell proliferation and promote migration in wound healing. *Pharmaceutics* 2020; 12: 389.
68 Baker DJ, Wijshake T, Tchkonia T, *et al.* Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 2011; 479: 232–236.
69 Schafer MJ, White TA, Iijima K, *et al.* Cellular senescence mediates fibrotic pulmonary disease. *Nat Commun* 2017; 8: 14532.
70 Del Principe D, Vona R, Giordani L, *et al.* Defective autophagy in fibroblasts may contribute to fibrogenesis in autoimmune processes. *Curr Pharm Des* 2011; 17: 3878–3887.
71 Zhao H, Wang Y, Qiu T, *et al.* Autophagy, an important therapeutic target for pulmonary fibrosis diseases. *Clin Chim Acta* 2020; 502: 139–147.
72 Hill C, Li J, Liu D, *et al.* Autophagy inhibition-mediated epithelial-mesenchymal transition augments local myofibroblast differentiation in pulmonary fibrosis. *Cell Death Dis* 2019; 10: 591.
73 Ghavami S, Yeganeh, B Zeki, AA, *et al.* Autophagy and the unfolded protein response promote profibrotic effects of TGF-β(1) in human lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 2018; 314: L493–L504.
74 Larson-Casey JL, Deshane JS, Ryan AJ, *et al.* Macrophage Akt1 kinase-mediated mitophagy modulates apoptosis resistance and pulmonary fibrosis. *Immunity* 2016; 44: 582–596.
75 Hecker L. Mechanisms and consequences of oxidative stress in lung disease: therapeutic implications for an aging populace. *Am J Physiol Lung Cell Mol Physiol* 2018; 314: L642–L653.
76 Kling KM, Lopez-Rodriguez E, Pfarrer C, *et al.* Aging exacerbates acute lung injury-induced changes of the air-blood barrier, lung function, and inflammation in the mouse. *Am J Physiol Lung Cell Mol Physiol* 2017; 312: L1–L12.
77 Lawson WE, Grant SW, Ambrosini V, *et al.* Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. *Thorax* 2004; 59: 977–980.
78 Seibold MA, Wise AL, Speer MC, *et al.* A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med* 2011; 364: 1503–1512.
79 Bodas M, Silverberg D, Walworth K, *et al.* Augmentation of S-nitrosoglutathione controls cigarette smoke-induced inflammatory-oxidative stress and chronic obstructive pulmonary disease–emphysema pathogenesis by restoring cystic fibrosis transmembrane conductance regulator function. *Antioxid Redox Signal* 2017; 27: 433–451.
80 Sosulski ML, Gongora R, Danchuk S, *et al.* Deregulation of selective autophagy during aging and pulmonary fibrosis: the role of TGFβ1. *Aging Cell* 2015; 14: 774–783.
81 Barkauskas CE, Cronce MJ, Rackley CR, *et al.* Type 2 alveolar cells are stem cells in adult lung. *J Clin Invest* 2013; 123: 3025–3036.
82 Ortega-Martínez M, Rodríguez-Flores LE, Ancer-Arellano A, *et al.* Analysis of cell turnover in the bronchiolar epithelium through the normal aging process. *Lung* 2016; 194: 581–587.
83 Watson JK, Sanders P, Dunmore R, *et al.* Distal lung epithelial progenitor cell function declines with age. *Sci Rep* 2020; 10: 10490.
84 Wansleeben C, Bowie E, Hotten DF, *et al.* Age-related changes in the cellular composition and epithelial organization of the mouse trachea. *PLoS One* 2014; 9: e93496.
85 Ryan BM, Wang Y, Jen J, *et al.* Evidence that the lung adenocarcinoma EML4-ALK fusion gene is not caused by exposure to secondhand tobacco smoke during childhood. *Cancer Epidemiol Biomarkers Prev* 2014; 23: 1432–1434.
86 Ghosh M, Ahmad S, White CW, *et al.* Transplantation of airway epithelial stem/progenitor cells: a future for cell-based therapy. *Am J Respir Cell Mol Biol* 2017; 56: 1–10.
87 Araya J, Kojima J, Takasaka N, *et al.* Insufficient autophagy in idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2013; 304: L56–L69.
88 Yu Y, Li W, Ren L, *et al.* Inhibition of autophagy enhanced cobalt chloride-induced apoptosis in rat alveolar type II epithelial cells. *Mol Med Rep* 2018; 18: 2124–2132.
89 Pastukh VM, Zhang L, Ruchko MV, *et al.* Oxidative DNA damage in lung tissue from patients with COPD is clustered in functionally significant sequences. *Int J Chron Obstruct Pulmon Dis* 2011; 6: 209–217.
90 Neofytou E, Tzortzaki EG, Chatziantoniou A, *et al.* DNA damage due to oxidative stress in chronic obstructive pulmonary disease (COPD). *Int J Mol Sci* 2012; 13: 16853–16864.
91 Jablonski RP, Kim SJ, Cheresh P, *et al.* SIRT3 deficiency promotes lung fibrosis by augmenting alveolar epithelial cell mitochondrial DNA damage and apoptosis. *FASEB J* 2017; 31: 2520–2532.
92 Yuan L, Chen Z, Song S, *et al.* p53 degradation by a coronavirus papain-like protease suppresses type I interferon signaling. *J Biol Chem* 2015; 290: 3172–3182.
93 Ou HL, Schumacher B. DNA damage responses and p53 in the aging process. *Blood* 2018; 131: 488–495.

94 Ma-Lauer Y, Carbajo-Lozoya J, Hein MY, *et al.* p53 down-regulates SARS coronavirus replication and is targeted by the SARS-unique domain and PLpro via E3 ubiquitin ligase RCHY1. *Proc Natl Acad Sci USA* 2016; 113: E5192–E5201.

95 Domkin V, Thelander L, Chabes A. Yeast DNA damage-inducible Rnr3 has a very low catalytic activity strongly stimulated after the formation of a cross-talking Rnr1/Rnr3 complex. *J Biol Chem* 2002; 277: 18574–18578.

96 Sharma A, Alswillah T, Singh K, *et al.* USP14 regulates DNA damage repair by targeting RNF168-dependent ubiquitination. *Autophagy* 2018; 14: 1976–1990.

97 Park C, Suh Y, Cuervo AM. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. *Nat Commun* 2015; 6: 6823.

98 Park YE, Hayashi YK, Bonne G, *et al.* Autophagic degradation of nuclear components in mammalian cells. *Autophagy* 2009; 5: 795–804.

99 Zhang Y, W Huang, Z Zheng, *et al.* Cigarette smoke-inactivated SIRT1 promotes autophagy-dependent senescence of alveolar epithelial type 2 cells to induce pulmonary fibrosis. *Free Radic Biol Med* 2021; 166: 116–127.

100 Liu Q, Wu J, Zhang X, *et al.* Circulating mitochondrial DNA-triggered autophagy dysfunction *via* STING underlies sepsis-related acute lung injury. *Cell Death Dis* 2021; 12: 673.

101 Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 2011; 333: 1109–1112.

102 Araya J, Tsubouchi K, Sato N, *et al.* PRKN-regulated mitophagy and cellular senescence during COPD pathogenesis. *Autophagy* 2019; 15: 510–526.

103 Salimi S, Hamlyn JM. COVID-19 and crosstalk with the hallmarks of aging. *J Gerontol A Biol Sci Med Sci* 2020; 75: e34–e41.

104 Schiavi A, Maglioni S, Palikaras K, *et al.* Iron-starvation-induced mitophagy mediates lifespan extension upon mitochondrial stress in *C. elegans*. *Curr Biol* 2015; 25: 1810–1822.

105 Kobayashi K, Araya J, Minagawa S, *et al.* Involvement of PARK2-mediated mitophagy in idiopathic pulmonary fibrosis pathogenesis. *J Immunol* 2016; 197: 504–516.

106 Coppé JP, Desprez PY, Krtolica A, *et al.* The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 2010; 5: 99–118.

107 Kuilman T, Peeper DS. Senescence-messaging secretome: SMS-ing cellular stress. *Nat Rev Cancer* 2009; 9: 81–94.

108 Gorgoulis V, Adams PD, Alimonti A, *et al.* Cellular senescence: defining a path forward. *Cell* 2019; 179: 813–827.

109 Xu J, Gonzalez ET, Iyer SS, *et al.* Use of senescence-accelerated mouse model in bleomycin-induced lung injury suggests that bone marrow-derived cells can alter the outcome of lung injury in aged mice. *J Gerontol A Biol Sci Med Sci* 2009; 64: 731–739.

110 Zhou F, Onizawa S, Nagai A, *et al.* Epithelial cell senescence impairs repair process and exacerbates inflammation after airway injury. *Respir Res* 2011; 12: 78.

111 Baker DJ, Childs BG, Durik M, *et al.* Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* 2016; 530: 184–189.

112 Hashimoto M, Asai A, Kawagishi H, *et al.* Elimination of p19(ARF)-expressing cells enhances pulmonary function in mice. *JCI Insight* 2016; 1: e87732.

113 Aoshiba K, Nagai A. Senescence hypothesis for the pathogenetic mechanism of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2009; 6: 596–601.

114 Wang ZN, Su RN, Yang BY, *et al.* Potential role of cellular senescence in asthma. *Front Cell Dev Biol* 2020; 8: 59.

115 Lee S, Yu Y, Trimpert J, *et al.* Virus-induced senescence is a driver and therapeutic target in COVID-19. *Nature* 2021; 599: 283–289.

116 Rajawat YS, Hilioti Z, Bossis I. Aging: central role for autophagy and the lysosomal degradative system. *Ageing Res Rev* 2009; 8: 199–213.

117 Morselli E, Maiuri MC, Markaki M, *et al.* The life span-prolonging effect of sirtuin-1 is mediated by autophagy. *Autophagy* 2010; 6: 186–188.

118 Lee IH, Cao L, Mostoslavsky R, *et al.* A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci USA* 2008; 105: 3374–3379.

119 Young AR, Narita M, Ferreira M, *et al.* Autophagy mediates the mitotic senescence transition. *Genes Dev* 2009; 23: 798–803.

120 Goehe RW, Bristol ML, Wilson EN, *et al.* Autophagy, senescence, and apoptosis. *Methods Mol Biol* 2013; 962: 31–48.

121 Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008; 454: 428–435.

122 Franceschi C, Garagnani P, Vitale G, *et al.* Inflammaging and 'Garb-aging'. *Trends Endocrinol Metab* 2017; 28: 199–212.

123 Meftahi GH, Jangravi Z, Sahraei H, *et al.* The possible pathophysiology mechanism of cytokine storm in elderly adults with COVID-19 infection: the contribution of 'inflame-aging'. *Inflamm Res* 2020; 69: 825–839.

124 Chen Y, Klein SL, Garibaldi BT, *et al.* Aging in COVID-19: vulnerability, immunity and intervention. *Ageing Res Rev* 2021; 65: 101205.

125 Zitvogel L, Kepp O, Kroemer G. Decoding cell death signals in inflammation and immunity. *Cell* 2010; 140: 798–804.

126 Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol* 2013; 13: 722–737.

127 Sun Q, Fan J, Billiar TR, *et al.* Inflammasome and autophagy regulation – a two-way street. *Mol Med* 2017; 23: 188–195.

128 Sohal RS, Allen RG. Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. *Exp Gerontol* 1990; 25: 499–522.

129 von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci* 2000; 908: 99–110.

130 Mizushima N. Physiological functions of autophagy. *Curr Top Microbiol Immunol* 2009; 335: 71–84.

131 Zhang X, Cheng X, Yu L, *et al.* MCOLN1 is a ROS sensor in lysosomes that regulates autophagy. *Nat Commun* 2016; 7: 12109.

132 Medina DL, Di Paola S, Peluso I, *et al.* Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat Cell Biol* 2015; 17: 288–299.

133 Zhang J, Kim J, Alexander A, *et al.* A tuberous sclerosis complex signalling node at the peroxisome regulates mTORC1 and autophagy in response to ROS. *Nat Cell Biol* 2013; 15: 1186–1196.

134 Bellot G, Garcia-Medina R, Gounon P, *et al.* Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L *via* their BH3 domains. *Mol Cell Biol* 2009; 29: 2570–2581.

135 Chandel NS, McClintock DS, Feliciano CE, *et al.* Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O_2 sensing. *J Biol Chem* 2000; 275: 25130–25138.

136 Mazure NM, Pouysségur J. Hypoxia-induced autophagy: cell death or cell survival? *Curr Opin Cell Biol* 2010; 22: 177–180.

137 Jiang Y, Wang X, Hu D. Mitochondrial alterations during oxidative stress in chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2017; 12: 1153–1162.

138 Aguilera MO, Delgui LR, Romano PS, *et al.* chronic infections: a possible scenario for autophagy and senescence cross-talk. *Cells* 2018; 7: 162.

139 He C, Bassik MC, Moresi V, *et al.* Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature* 2012; 481: 511–515.

140 Hannan MA, Rahman MA, Rahman MS, *et al.* Intermittent fasting, a possible priming tool for host defense against SARS-CoV-2 infection: crosstalk among calorie restriction, autophagy and immune response. *Immunol Lett* 2020; 226: 38–45.