Chapter 6
Pathogenesis of COPD 4 – Cell Death, Senescence, and Autophagy: Is There a Possibility of Developing New Drugs from the Standpoint of This Pathogenetic Mechanism?

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Abstract The balance of cell survival and death is essential feature of homeostasis. Apoptosis is a well-known form of cell death and involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). The number of apoptotic epithelial and endothelial cells is increased in lung tissues from patients with COPD. Protease-antiprotease imbalance and oxidative stress amplify alveolar destruction. Cellular senescence is one of the cellular stress responses and considered to be one of the processes of aging. COPD has been assumed to be a disease of accelerated lung aging, and cellular senescence has been widely implicated in the pathogenesis of COPD, presumably by impairing cell repopulation and by the aberrant cytokine secretion seen in senescence-associated secretory phenotype, which may exert deleterious effects on the tissue microenvironment of neighboring cells. Autophagy is a process of lysosomal self-degradation that helps maintain homeostatic balance between the synthesis, degradation, and recycling of cellular proteins and organelles. Autophagy is closely related with aging, since autophagy diminishes with aging, and accelerated aging can be attributed to reduced autophagy. Insufficient autophagic clearance is reported to be involved in accelerated cellular senescence in COPD, while excessive autophagy might induce epithelial cell necroptosis in COPD. The detailed molecular mechanism for regulation of autophagy and cellular senescence is complex, and the role of autophagy and cellular senescence is overlapped significantly. We review molecular mechanisms of cell death, cellular

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senescence, and autophagy and summarize the role of these stress responses in the pathogenesis of COPD.

Keywords Apoptosis • Aging • Senescence • Autophagy • Chronic obstructive pulmonary disease (COPD)

6.1 The Role of Cell Death in the Pathophysiology of COPD

6.1.1 Apoptosis

The balance of cell survival and death is an essential feature of homeostasis. Apoptosis and necrosis are a well-known form of cell death and involved in various human diseases. Recently, other forms of cell death, such as caspase-independent cell death, oncosis, and autophagy-associated cell death, have also been investigated in various diseases. Apoptosis is characterized by cell shrinkage, apoptotic bodies, and intact cellular membranes. Biochemical markers of apoptosis are caspase activation, DNA fragmentation, and externalization of phosphatidylserine. There are two principle-signaling pathways of apoptosis. One is a direct pathway, named the extrinsic pathway, from death receptor ligation to caspase cascade activation and cell death. Death receptor ligation triggers recruitment of the precursor form of caspase-8 to a death-inducing complex, through the adaptor protein Fas-associating protein with death domain (FADD), which leads to caspase-8 activation. The other pathway, named the intrinsic pathway, triggered by stimuli such as drugs, radiation, infectious agents, and reactive oxygen species (ROS), is initiated in mitochondria. Mitochondrial outer membrane permeability is regulated by Bcl-2 family proteins. After cytochrome c is released into the cytosol from the mitochondria triggered by various stimuli, it binds to Apaf1 and ATP, which then activate caspase-9 [1]. The activation of initiator caspase-8 and caspase-9 resulted in activation of effecter caspases such as caspase-3. Active executioner caspases mediate the cleavage of protein substrates, resulting in morphological features of apoptosis. Recently, endoplasmic reticulum has also been shown to be the organelle to execute apoptosis. Various stresses can impair protein folding and induce endoplasmic reticulum stress, and severe endoplasmic reticulum stress can transduce apoptotic signals [2].

6.1.2 Cell Death and COPD

Increased numbers of apoptotic alveolar, bronchiolar, and endothelial cells in lung tissues from patients with COPD were demonstrated in several reports. Particles, xenobiotics, and oxidants contained in cigarette smoke induce inflammation, oxidative stress, and protease activation. Protease-antiprotease imbalance and oxidative stress amplify alveolar destruction [3]. Since a large number of lymph follicles

are found in lung tissues from patients with COPD, T-cell-mediated immune response is also considered to have a role in inducing apoptosis [4].

Pulmonary emphysema is characterized by the enlargement of distal air spaces due to the destruction and loss of alveolar structures. Recently, endothelial and epithelial cell apoptosis have been implicated as one of important mechanisms of pulmonary emphysema. Intratracheal injection of activated caspase-3 induces epithelial cell apoptosis, enhances elastolytic activity, and subsequently induces emphysematous changes in mice [5]. In addition, overexpression of α-1 antitrypsin attenuated endothelial cell death, alveolar wall destruction, and oxidative stress caused by caspase-3 instillation. In TNF-α and IL-1β receptor deficient mice, the degree of emphysematous changes and lung cell apoptosis are decreased after intratracheal instillation of elastase. Therefore, inflammation and protease activation accelerate epithelial, endothelial cell apoptosis and emphysema.

Cigarette smoke induces epithelial cell apoptosis, activated caspases, protease activation, and chemokines via IL-18Rα-dependent manner. Cigarette smoke induces mitochondrial dysfunction by blocking mitochondrial respiratory chain, loss of ATP generation, which leads to cellular necrosis rather than apoptosis [6]. The mitochondrial localization of HO-1 in bronchiolar epithelial cells was confirmed using electron microscopy. Overexpression of HO-1 inhibited cigarette smoke extract (CSE)-induced cell death. As well as cigarette smoke, air pollution contains high levels of nitrogen/oxygen species and reactive oxygen species, which activate different sphingomyelinases to induce apoptosis in airway epithelial cells.

Chronic treatment of rats with the vascular endothelial growth factor (VEGF) receptor blocker induces alveolar cell apoptosis, which subsequently leads to enlargement of the alveolar spaces without inflammatory cell infiltration or fibrosis [7]. VEGF and VEGF receptor type II expressions are also decreased in lung tissues from patients with pulmonary emphysema compared with controls. These results suggest that the defect of alveolar wall maintenance factors may lead to alveolar cell apoptosis.

Apoptotic cells should be removed rapidly by phagocytosis also called "efferocytosis" for the resolution of inflammation without damaging the tissues. Defective removal of apoptotic cells as well as more apoptosis is thought to be important as a contributor to COPD [8]. Surfactant protein (SP)-D deficient mice accumulate apoptotic macrophages in the lung, and exogenous SP-D binds to apoptotic macrophages. These results suggest that SP-D may have an important role in the clearance of apoptotic cells and have preventive effects on the development of emphysema.

In lung tissues from patients with emphysema, alveolar cell apoptosis and expression of PCNA in epithelium are increased, which suggests the activation of regenerative processes. However, cigarette smoke extract induces oxidative stress and apoptosis not only epithelium but also lung fibroblast and impairs repair processes [9]. Since apoptosis is likely to be involved in not only the destructive phase but also remodeling process, regulating apoptosis may become an effective treatment against COPD. Concerning regulating apoptosis in COPD, cellular senescence and autophagy are also involved, although its mechanisms are not well understood.

6.2 The Role of Cellular Senescence in the Pathophysiology of COPD

6.2.1 Aging and Cellular Senescence

Aging is associated with the impaired function of maintaining homeostasis in organs and bodies. The phenotypes of aging include genomic instability, telomere erosion, epigenetic changes, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered cellular communication [10]. Hayflick et al. firstly used the term "replicative senescence" to describe phenomenon of irreversible growth arrest of normal human fibroblasts after extensive serial passaging in culture [11]. Replicative senescence was caused by telomere shortening. Senescent cells in tissues have usually been identified using histological staining for DNA damage markers such as p21, p16, or senescent-associated β-galactosidase (SA-βgal) activity. In the liver, skin, lung, and spleen, a total of ~8 % and ~17 % senescent cells in young and old mice were identified, respectively, although there was no change in the heart, skeletal muscle, and kidney. Therefore, cellular senescence is not a generalized property of aged tissues, and aging and senescence are not equal. As the first identification of cellular senescence in lung diseases, the positive staining for senescence-associated heterochromatin foci marker γH2AX in the alveolar epithelial cells of old mice was demonstrated. In human lung tissue, human lung fibroblasts obtained from lung tissues from patients with COPD showed reduced proliferation rate compared with those from healthy lung. Cellular senescence is induced by not only telomere shortening but various cellular stresses such as oxidative stress, oncogene activation, DNA damage, and chromatin abnormality [12]. In addition, it is noteworthy that cellular senescence also plays instructive roles in organ and tissue development [13].

The characteristics of senescent cells include irreversible growth arrest, enlarged morphology, expression of cyclin-dependent kinase inhibitor (CDKI), the formation of senescence-associated heterochromatin foci, and senescence-associated secretory phenotype (SASP). CDKIs, such as p21 and p16, control cell cycling. The increased expression of CDKIs results in cell cycle arrest in senescent cells. Senescent cells affect microenvironment through gene expression of growth factors, cytokines, and proteases, so-called SASP. SASP presents biological activities and plays a key role in diverse effects on carcinogenesis and the pathogenesis of degenerative diseases [14]. Senescent cells increase in size in vitro but not in vivo, enlarging sometimes as double as non-senescent cells. The markers of senescent cells include positive staining for SA-βgal which reflects the increase of lysosome contents; senescence-associated CDKIs p21, p16, p15, and p27 expression; and senescence-associated heterochromatin foci which inhibit gene expression of cell proliferation. These markers are not entirely specific to cellular senescence; therefore, cellular senescence has been defined by a collection of these markers. The

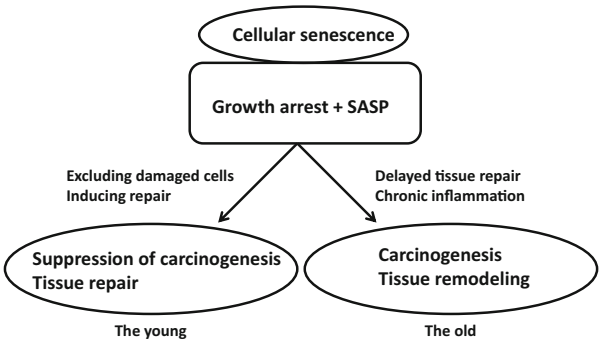

Fig. 6.1 The role of cellular senescence. Cellular senescence plays roles in tissue repair and carcinogenesis. SASP secreted from senescent cells stimulates the migration of phagocytic immune cells which play important roles in the clearance of senescent cells and the repair or resolution of damaged tissues. The tissue damage is prolonged when damaged tissue is not normally repaired. The prolongation of damaged tissues can lead to the accumulation of senescent cells. Senescent cells accumulate, secrete SASP, and induce remodeling of damaged tissues or proliferation of tumor cells in the old

phenotype of cellular senescence is various depending on the type of cell, senescent stimuli, and SASP [14].

Cellular senescence plays roles in tissue repair and regeneration [15]. SASP secreted from senescent cells stimulates the migration of phagocytic immune cells which play important roles in the clearance of senescent cells and the repair or resolution of damaged tissues. The tissue damage is prolonged when damaged tissue is not normally repaired or resolved. The prolongation of damaged tissues can lead to the accumulation of senescent cells. Therefore, senescent cells accumulate and secreted proteins, and other factors induce remodeling of damaged tissues or proliferation of tumor cells in the old [16] (Fig. 6.1). Recently, senescence has been reported to play important roles in the development processes and to compensate the role of apoptosis to remove unnecessary cells [13].

6.2.2 Cellular Senescence and COPD

COPD has been assumed to be a disease of accelerated lung aging, and cellular senescence has been widely implicated in the pathogenesis of COPD, presumably by impairing cell repopulation and by the aberrant cytokine secretion seen in SASP [17, 18]. Telomere length of neutrophils of COPD is shorter than that of healthy controls. Cellular senescence is found in lung epithelial cells, endothelial cells, and fibroblasts in patients with COPD [17, 19].

Sirtuin family belongs to class III histone deacetylases (HDAC), and one of sirtuin family SIRT1 has been extensively studied and well known as an antiaging molecule because of SIRT1-mediated longevity by calorie restriction [20]. SIRT1

expression is decreased in the lung tissues from patients with COPD by oxidative stress and smoking [21]. Decreased SIRT1 expression results in the increased expression of proinflammatory cytokines due to NF-kB activation and also results in the acceleration of cellular senescence mediated by the decrease of anti-senescent activity through FOXO3. Cellular senescence and emphysema were suppressed in SIRT1 transgenic mice by a FOXO3-mediated reduction of premature senescence in mice, while those are deteriorated in SITR1 knockout mice. SIRT1 activator SRT1720 suppressed emphysematous change in mice lung induced by elastase instillation and smoking inhalation [22].

6.3 The Role of Autophagy in the Pathophysiology of COPD

6.3.1 Autophagy

Autophagy is a process of lysosomal self-degradation that helps maintain homeostatic balance between the synthesis, degradation, and recycling of cellular proteins and organelles [23]. At the cellular level, autodigestion takes place within lysosomes and proteasomes. Proteasomes are involved in the clearance of ubiquitin-conjugated soluble proteins, whereas autophagy delivers diverse cytoplasmic components to the lysosome, including soluble proteins, aggregate-prone proteins, and organelles [24]. Autophagy is not simply machinery for amino acid supply in response to energy demand; it is an adaptive pathway of cytoprotection from cellular stresses, involving starvation, reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, and microbe infection [24].

Three forms of distinct autophagy have been demonstrated: chaperon (Hsc70)-mediated autophagy (CMA), microautophagy, and macroautophagy. CMA degrades soluble proteins only, via direct translocation to the lysosome through Lamp2A, a lysosomal transmembrane protein. During microautophagy, small components of the cytoplasm are engulfed by direct invagination into lysosomes. Engulfment of cytoplasmic components by the isolation membrane (phagophore) is the initial step in autophagy and is followed by elongation and fusion, resulting in formation of double-membranous vesicles (autophagosome). Subsequent fusion of the autophagosome with the lysosome to form the autolysosome is absolutely required for proper degradation [25]. Recent advances in the molecular mechanisms of autophagy have mainly focused on macroautophagy, specifically on the detection of a series of autophagy-related (*Atg*) genes. Among 35 autophagy-related, (Atg), proteins identified in yeast, there are core Atg proteins required for autophagosome formation, which are well conserved in mammals. Hence, in general in the literature, macroautophagy is designated as autophagy [25].

Initially, autophagy was proposed to be a nonselective bulk degradation system, but recent advances demonstrate that a variety of ubiquitinated cargos, including

protein aggregates, mitochondria, and microbes, are selective targets for autophagic degradation [25]. Accordingly, ubiquitination is an important tag for not only proteasomal degradation but also for selective autophagy. The p62 protein / sequestosome 1 (SQSTM1) has been shown to be an adaptor protein for selective autophagy based on its ability to bind both ubiquitin and microtubule-associated protein 1A/1B-light chain 3 (LC3), a crucial component for autophagosome formation [26]. Because of the dynamic nature of autophagy, in which autophagosome can be formed within several minutes, it is difficult to distinguish between increased autophagy flux and impaired subsequent clearance when using electron microscopic detection of autophagosomes or when examining Atg expression levels. Therefore, to detect the conversion of LC3-I to LC3-II (which is conjugated to phosphatidylethanolamine (PE) to ensure stable association to the autophagosomal membrane), the use of protease inhibitors is generally accepted to be standard methodology for evaluation of autophagy flux. In addition, based on the findings of selective autophagic degradation, concomitant accumulation of p62 and ubiquitinated protein is also recognized to at least partly reflect autophagy activity.

Due to the large number of physiological and aberrant intracellular components that are potential targets for autophagic degradation, autophagy status is linked to a diverse array of cellular processes and cell fates, including energy supply, homeostatic turnover of organelles, cell fate, cellular senescence, and immune responses [23]. In terms of the pathogenic role of autophagy, excessive activation may be associated with disease progression in extraphysiologic condition, whereas impairment of autophagy activity has been widely implicated in the pathogenic sequence of a variety of human disorders [26]. Indeed, recent in vitro and in vivo gene knockout studies revealed that insufficient autophagy is involved in the development of lung diseases [27–30].

Continuous ventilation of large amounts of air with high oxygen concentration, which may contain noxious particles and harmful microbes, is a fundamental function of the lungs and is required for sufficient gas exchange. Subsequently, indicating lung cells are serially exposed to a diverse array of cellular stresses, and it is reasonable to speculate that autophagy-mediated alleviation of cellular stress plays a key regulatory role in lung pathophysiology.

6.3.2 Autophagy and Cell Death

Apoptosis is necessary for the maintenance of homeostatic plasticity in the lung. However, the cell-type-specific imbalance of positive and negative regulation of apoptosis has been proposed to be a critical determination of lung disease progression [31]. Although autophagy had been postulated to be responsible for type II programmed cell death, current understanding is mainly that autophagic cell death is attributable simply to overwhelming autophagosome formation as a part of stress responses, in which cytosol and organelles are destroyed to an unrecoverable degree. Autophagy is an adaptation pathway for cellular stress, including

starvation, ROS, endoplasmic reticulum (ER) stress, and microbe infection; hence autophagy is generally considered to be a mechanism for cell survival. However, *Atg*-gene-dependent cell death has been reported in the setting of dysfunction of apoptosis machinery. Indeed, double knockout of Bax/Bak, essential components of the mitochondrial apoptotic pathway, demonstrated a distinct type of cell death, marked by accumulation of autophagosomes [32]. *Atg5* knockdown ameliorated this cell death, indicating that autophagy may promote cell death in the setting of extraphysiologic apoptosis deficiency.

In addition, there is functional cross talk between apoptosis and autophagy. For instance, B-cell lymphoma-2 (Bcl-2), an anti-apoptotic protein, interferes with starvation-dependent autophagy by binding to Beclin1 (Atg6) [33], which is an important constituent of the class III phosphatidylinositol (Ptdlns) 3-kinase complex involved in nucleation and assembly of the initial phagophore membrane. During receptor-mediated apoptosis, cleavage of Atg3 protein by caspase-8- suppresses autophagy activation, resulting in increased cell death. Furthermore, irrespective of their known role in autophagy induction, several Atg proteins may exert diverse regulatory roles during both the apoptotic process and cell survival. Fragmented Atg5 cleaved by calpain promotes intrinsic mitochondrial apoptosis [34]. Atg5 on the autophagosomal membrane serves as a platform for an intracellular death-inducing signaling complex (iDISC) that recruits caspase-8 to initiate the apoptosis cascade [35]. LC3B has been demonstrated to regulate apoptosis through interaction with Fas and caveolin-1(Cav-1). Therefore, clarification of whether the autophagy process is involved in apoptosis regulation is a prerequisite for proper interpretation of experimental results in the setting of Atg manipulation.

6.3.3 Autophagy and Cellular Senescence

Cellular senescence has been widely implicated in disease pathogenesis in terms of not only impaired cell repopulation but also aberrant cytokine secretions of SASP. SASP may exert deleterious effects on the tissue microenvironment of neighboring cell [36]. Increased cellular senescence is one of major features of aging, and hence cellular senescence has been widely implicated in age-associated disorders. The detailed molecular mechanism for regulation of cellular senescence is complex and incompletely understood, but one of the typical manifestations is accumulation of damaged proteins and organelles, occasionally associated with ubiquitinated aggregations [37]. Therefore, it has been proposed that functional insufficiencies in the cellular cleaning and housekeeping mechanisms of autophagy play a pivotal role in the accumulation of deleterious cellular components and therefore in the regulation of cellular senescence [37].

Indeed, autophagy diminishes with aging, and accelerated aging can be attributed to reduced autophagy. Thus, autophagy activation appears to be associated with longevity [38]. Pathologic premature aging due to autophagy malfunction has been intensively examined using animal models of autophagy inhibition by

tissue-specific knockout of *Atg* genes. Those animal models with insufficient autophagy demonstrated a cellular phenotype of progressive accumulation of ubiquitinated aggregates and disorganized mitochondria, suggesting the causal relationship between loss of autophagy and aging-associated disease phenotypes [38]. However, those phenotypic alterations were mainly evaluated in the central nervous system and liver, not in other organs. Among the variety of targets for autophagic degradation, selective autophagy of mitochondria (mitophagy) has been widely implicated in cellular senescence in terms of regulation of reactive oxygen species (ROS) of oxidative stress. Mitochondria are the main organelle responsible for intrinsic ROS release through respiratory chain reactions, and insufficient mitophagy results in accumulation of damaged mitochondria accompanied by increased ROS production [39].

The role of stress-induced autophagy activation in longevity has been mainly demonstrated in the case of caloric restriction (CR) [40]. CR induces autophagy through the inhibition of mammalian target of rapamycin (mTOR), an essential negative regulator of autophagy, and also through activation of adenosine monophosphate-activated protein kinase (AMPK) and sirtuin1 (SIRT1). In response to the rising AMP/ATP ratio during CR, AMPK induces autophagy via phosphorylation of ULK1, a mammalian orthologue of the yeast protein kinase Atg1. SIRT1 deacetylation of Atg proteins and transcription factors, including the FOXO family, is involved in autophagy induction [22]. The involvement of CR-induced autophagy in longevity was confirmed by inhibition of autophagy, and SIRT1-mediated longevity by CR is at least partly conferred by autophagy activation. Intriguingly, recent paper demonstrated that SIRT1 protects against emphysema by a FOXO3-mediated reduction of premature senescence in mice, but the involvement of autophagy was not examined.

Autophagy is getting more importance during the aging process, because proteasome pathway could not degrade protein aggregates in the presence of an enhanced prooxidant and aggregation-prone milieu characteristic of aging [41]. Pharmacological inhibition of autophagy prevented development of premature senescence but did lead to the enhanced rate of apoptosis in human umbilical vein endothelial cells. A subset of autophagy-related genes is upregulated during senescence [42]. Overexpression of one of those genes, ULK3, induces autophagy and senescence. Furthermore, inhibition of autophagy delays the senescence phenotype, including senescence-associated secretion [43]. Goehe et al. showed that autophagy and senescence tend to occur in parallel and furthermore that autophagy accelerates the development of the senescent phenotype [44]. Collectively, autophagy and cellular senescence are associated with each other in some situations, but these two important cellular processes may be interdependently involved in the pathophysiology of several diseases.

6.3.4 Autophagy and COPD

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death worldwide and is characterized by partially irreversible and progressive airflow limitation. Cigarette smoke, the major cause of COPD, is rich in toxic components including ROS, and a variety of biological responses to cigarette smoke exposure have been demonstrated. Although detailed molecular mechanisms for COPD development remain unclear, the possible participation of autophagy in the pathogenic sequence of COPD has been intensively explored (Fig. 6.2). It has been reported that autophagy in lung tissue from COPD patients is augmented by means of an increase in the LC3B-II/LC3B-I ratio, and Egr-1-induced LC3B expression is essential for autophagy activation [45]. LC3B−/− mouse experiments confirmed the pivotal role of LC3B in epithelial cell apoptosis induction by cigarette smoke exposure. The proposed mechanism of LC3B-induced apoptosis is attributed to the balance in a trimolecular interaction between LC3B with Fas and caveolin-1(Cav-1), a lipid raft protein. LC3B knockdown inhibits apoptosis by increasing Cav-1-dependent Fas sequestration, and dissociation of Fas and LC3B from Cav-1 in response to CSE exposure initiates apoptosis in epithelial cells. LC3B is a key component for autophagy machinery, and association between LC3B and Fas is an interesting observation; however it is still unclear whether autophagy activation by LC3B expression is crucial for apoptosis induction in these COPD models. Furthermore, in cases of hyperoxia-induced apoptosis in epithelial cells, LC3B interacts with Fas, resulting in prevention of apoptosis [46], suggesting that the role of association between LC3B and Fas in apoptosis regulation is dependent on the stimuli or experimental conditions. Intriguingly, decreased autophagy activity in alveolar macrophages derived from smokers has been reported in terms of impaired xenophagy. In spite of increased LC3B-II and autophagosomes in macrophages from smokers, impairment of autophagy flux was shown using protease inhibitors and also by detecting accumulation of p62 aggregates [47], indicating that autophagy activity in COPD lung may be regulated via cell-type-specific mechanisms.

Autophagy plays a pivotal regulatory role for cellular senescence; hence we have attempted to elucidate the involvement of autophagy in the regulation of cigarette smoke extract (CSE)-induced human bronchial epithelial cell (HBEC) senescence [27]. CSE transiently induces autophagy activation followed by accumulations of p62 and ubiquitinated proteins accompanied by an increase in HBEC senescence. Autophagy inhibition by 3MA, a specific inhibitor of autophagic sequestration, or by LC3B and Atg5 knockdown further enhanced HBEC senescence with concomitant accumulation of p62 and ubiquitinated proteins [27]. In contrast, autophagy activation by Torin1, a mammalian target of rapamycin (mTOR) inhibitor, suppressed p62 and ubiquitinated protein accumulations and also inhibited HBEC senescence. In line with the previous finding of increased autophagy activation in COPD epithelial cells, we observed an increase in baseline autophagy but also found significantly decreased autophagy induction in response to CSE exposure in

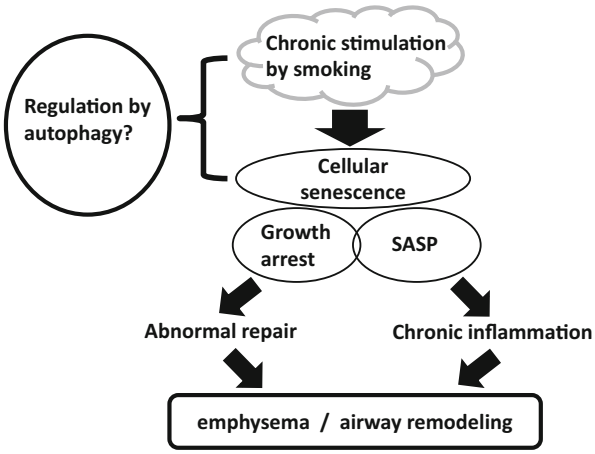

Fig. 6.2 The pathophysiology of COPD. Cigarette smoke, the major cause of COPD, is rich in toxic components including ROS, and a variety of biological responses to cigarette smoke exposure including cellular senescence has been demonstrated. Abnormal repair and SASP induce alveolar wall destruction and small airway remodeling. Although detailed molecular mechanisms for COPD development remain unclear, the possible participation of autophagy in the pathogenic sequence of COPD has been intensively explored

HBEC isolated from COPD patients compared to those from nonsmokers [27]. We speculated that the mechanism for enhanced baseline autophagy flux was attributed to increased oxidative stress, which was demonstrated by the accumulation of carbonylated proteins in HBEC from COPD patients [18]. Therefore, it is probable that the attenuation of autophagy flux in response to CSE exposure may reflect an insufficient reserve of autophagy activation in HBEC from COPD patients. Concomitant accumulation of p62 and ubiquitinated protein is also recognized to at least partly reflect autophagy activity. Increased accumulations of p62 and ubiquitinated proteins detected in lung homogenates support the notion that insufficient autophagic clearance is involved in accelerated cellular senescence in COPD [27] (Fig. 6.3).

SIRT6 has been demonstrated to regulate longevity by modulating insulin-like growth factor (IGF)-I signaling. IGF-I signaling activates mTOR, and a recent paper demonstrated that IGF-I exposure was sufficient to induce cellular senescence through inhibition of baseline autophagy [48]. Intriguingly, we demonstrated that CSE decreased the SIRT6 expression in HBEC and that CSE-induced HBEC senescence was inhibited by SIRT6 overexpression and that histone deacetylase (HDAC) activity of SIRT6 was indispensable for inhibition of CSE-induced HBEC senescence through autophagy activation, which was mainly attributed to attenuation of IGF-Akt-mTOR signaling [49]. Decreased expression levels of SIRT6 found in lung homogenates from COPD patients support the hypothesis that reduced SIRT6 expression with accompanying autophagy insufficiency may be associated with COPD development through the enhancement of cellular senescence, especially in the setting of increased IGF signaling. Furthermore, Decreased SIRT6

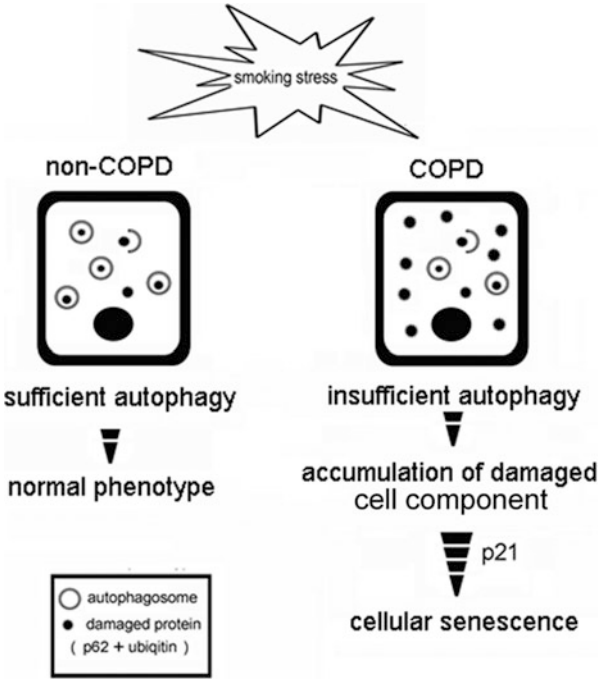

Fig. 6.3 Insufficient autophagic degradation in COPD pathogenesis. We found that baseline autophagy was increased, while autophagy induction was significantly decreased in response to CSE exposure in HBEC isolated from COPD patients compared to those from nonsmokers. We speculate that the mechanism for enhanced baseline autophagy flux may be attributed to increased oxidative stress. It is probable that the attenuation of autophagy flux in response to CSE exposure may reflect an insufficient reserve of autophagy activation in HBEC from COPD patients

expression was significantly correlated with the decrease of FEV_1% [49]. As IGF-I shares receptors and signaling pathways with insulin, and type II diabetes mellitus with hyperinsulinemia is a common comorbidity in COPD, it may be associated with COPD development via increased IGF/insulin signaling and autophagy inhibition, especially in cases of decreased SIRT6 expression.

Mitochondria are the main organelle producing ATP as well as reactive oxygen species (ROS) and play central roles in cell fate regulation. Mitochondria also release mitochondrial DNA as one of damage-associated molecular pattern. Therefore, maintenance of mitochondrial homeostasis is prerequisite for cellular homeostasis [50]. Mitochondria are dynamic organelles, which continuously change their shape through fission and fusion. Damaged and fragmented mitochondria are removed through mitochondria-specific autophagic degradation (mitophagy). Disruption of mitochondrial dynamics is involved in disease pathology through excessive reactive oxygen species (ROS) production [51] (Fig. 6.4). In electron microscopic examination of lung tissues, we demonstrated that mitochondria in bronchial epithelial cells tended to be fragmented in COPD, suggesting the fission process dominancy of mitochondrial dynamics in COPD pathogenesis [52]. In vitro studies further confirmed that CSE-induced excessive fragmentation of mitochondria is associated with mitochondrial ROS production, resulting in HBEC senescence. Autophagy inducer Torin1 accelerate degradation of damaged mitochondria in autophagosome, resulted in the increase of healthy mitochondria [52].

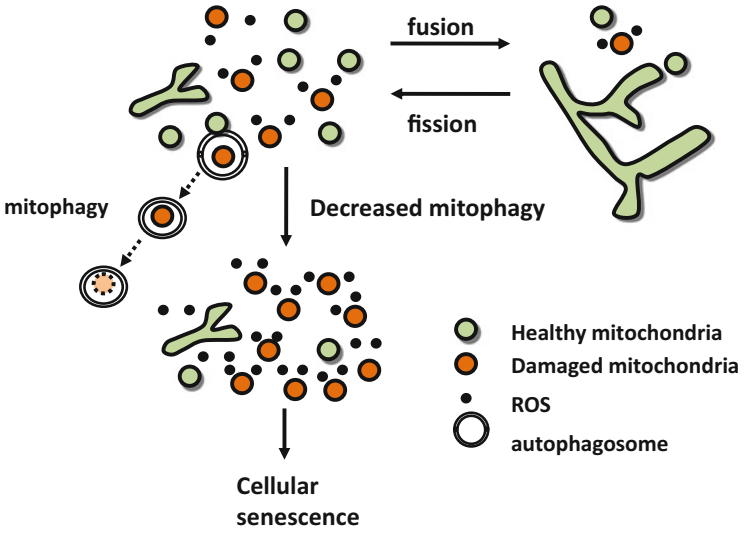

Fig. 6.4 The prevention of cellular senescence by mitophagy through excluding damaged mitochondria. Mitochondria are dynamic organelles, which continuously change their shape through fission and fusion. Damaged and fragmented mitochondria are removed through mitochondria-specific autophagic degradation (mitophagy). Disruption of mitochondrial dynamics is involved in disease pathology through excessive reactive oxygen species (ROS) production

The phosphatase and tensin homolog (PTEN)-induced putative protein kinase 1 (PINK1)-PARK2 pathway has been largely implicated in the removal of damaged mitochondria with depolarized membranes. Stress-induced membrane depolarization stabilizes PINK1, resulting in recruitment of PARK2, an E3 ubiquitin ligase, to mitochondria [53, 54]. We found that PARK2-mediated ubiquitination is crucial for mitophagic degradation in damaged mitochondria in HBEC. Knockdown of PINK1 or PARK2 decreased autophagy activation, the accumulation of damaged mitochondria accompanied by increased ROS production, and cellular senescence in HBEC [55]. PARK2 expression in lung tissue from patients with COPD was significantly decreased compared with that from smokers without COPD. The decreased PARK2 expression was significantly correlated with the decrease of FEV_1%. Immunohistochemistry results showed the expression of PARK2 in bronchial epithelial cells from patients with COPD was significantly decreased compared with that from nonsmokers or smokers without COPD. Therefore, the decrease of PARK2 expression may be associated with the deficiency of mitophagy and cellular senescence in COPD pathogenesis [55] (Fig. 6.5).

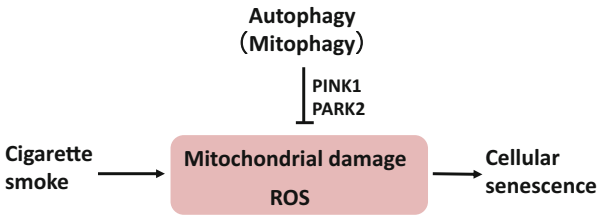

Fig. 6.5 The regulation of damaged mitochondrial removal and ROS production by mitophagy. Cigarette smoke induces mitochondrial damage in association with increased ROS production. This mitochondrial ROS is at least partly involved in cellular senescence. Autophagy suppresses accumulation of damaged mitochondria and ROS production, suggesting that mitochondria-specific autophagy (mitophagy) may play a pivotal role through PINK1 and PARK2 in the regulation of CSE-induced cellular senescence

6.4 Conclusions

An aging society has a problem with various diseases associated with aging. In particular, lung diseases have much attention because COPD, pneumonia, and lung cancer are speculated to be third, fourth, and fifth leading cause of death in the world, respectively. Increased numbers of apoptotic alveolar, bronchiolar, and endothelial cells in lung tissues from patients with COPD were demonstrated. Cigarette smoke induces inflammation, oxidative stress, and protease activation. Protease-antiprotease imbalance and oxidative stress amplify alveolar destruction through inducing apoptosis.

Cellular senescence is the most closely associated with aging processes; therefore, therapies targeting cellular senescence should be important strategies against COPD. Classic "free radical hypothesis" means that ROS induces cellular senescence. Many reports have shown that mitochondrial dysfunction leads to cellular senescence due to excessive ROS production. The treatment strategy against cellular senescence through induction of autophagy, especially mitophagy, may be promising against COPD.

Autophagy is responsible not just for simple homeostatic energy supply but also for elimination of aggregate-prone proteins, damaged organelles, and intracellular microbes. Autophagy is a dynamic process and may rapidly change its status, which can be influenced by not only disease activity but also environmental stresses. Additionally, the regulatory role of autophagy can be dependent on stages in disease development, and the pathogenic involvement may be different in a cell-type-specific manner.

Recent advances in cell death, senescence, and autophagy shed more light on understanding the pathogenesis of COPD and may lead to the development of new therapeutic options. The development of proper biomarkers reflecting status of senescence and autophagy is warranted to precisely evaluate disease progression, and the establishment of novel therapeutic approaches is also warranted to achieve optimal levels of cellular senescence and autophagy status.

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