

Autophagy and Cellular Senescence in Lung Diseases

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ABSTRACT

Autophagy is a process of lysosomal self-degradation that helps maintain homeostatic balance between the synthesis, degradation and recycling of cellular proteins and organelles. In addition to nutrient starvation, a wide array of cellular stresses are also known to be strong inducers of autophagy, indicating that autophagy is not only simple amino acid supply machinery in response to energy demand but also a central component of the integrated stress response for cytoprotection. Since autophagy is an adaptive pathway of cytoprotection from cellular stresses, involving starvation, reactive oxygen species, endoplasmic reticulum stress, and microbe infection, it is reasonable to suggest that autophagy is closely related with aging. Indeed, autophagy diminishes with aging and accelerated aging can be attributed to reduced autophagy. Cellular senescence is also one of the cellular stress responses as well as autophagy, and considered to be one of the processes of aging. Cellular senescence has been widely implicated in disease pathogenesis in terms of not only impaired cell repopulation but also aberrant cytokine secretions of senescence associated secretory phenotype, which may exert deleterious effects on the tissue microenvironment of neighboring

cells. The detailed molecular mechanism for regulation of autophagy and cellular senescence is complex and the role of autophagy and cellular senescence is overlap significantly. We review molecular mechanisms of autophagy and cellular senescence, and summarize the role of autophagy and cellular senescence in pulmonary disease pathogenesis.

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Key words: Autophagy; Senescence; Aging; Infection; Lung cancer; Bronchial asthma; Chronic obstructive lung disease (COPD); Idiopathic pulmonary fibrosis (IPF)

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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^[1]. At the cellular level, auto-digestion 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^[2]. 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^[2].

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^[3]. 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^[4]. Hence, in general in the literature, macroautophagy is designated as autophagy^[3].

Initially, autophagy was proposed to be a non-selective 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^[3]. 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^[4]. 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^[5].

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^[1]. In terms of the pathogenic role of autophagy, excessive activation may be associated with disease progression in extraphysiologic conditions^[6], whereas impairment of autophagy activity has been widely implicated in the pathogenic sequence of a variety of human disorders^[7]. Indeed, recent *in vitro* and *in vivo* gene knockout studies revealed that insufficient autophagy is involved in the development of lung diseases^[8-11].

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.

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^[12]. 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^[13]. 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- β gal) activity. In the liver, skin, lung, and spleen, total of ~8% and ~17% senescent cells in young and old mice were identified respectively, although there was no change in heart, skeletal muscle, and kidney^[14]. Therefore, cellular senescence is not a generalized property of aged tissues, and aging and senescence is not equal. As the first identification of cellular senescence in lung diseases, Wang *et al* demonstrated positive staining for senescence associated heterochromatin foci marker γ H2AX in the alveolar epithelial cells of old mice^[14]. In human lung tissue, Holz *et al.* found that human lung fibroblasts obtained from lung tissues from patients with COPD showed reduced proliferation rate compared with those from healthy lung^[15]. Cellular senescence is induced by not only telomere shortening but various cellular stresses such as oxidative stress, oncogene activation, DNA damage, and chromatin abnormality^[16]. In addition, it is noteworthy that cellular senescence also plays instructive roles in organ and tissue development^[17].

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)^[18]. CDKIs, such as p21 and p16, control cell cycling. The increased expression of CDKIs results in cell cycle arrest in senescent cells^[19,20]. 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^[21]. 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- β gal 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 phenotype of cellular senescence is various depending on the type of cell, senescent stimuli, and SASP^[22] (Figure 1).

Cellular senescence plays roles in tissue repair and regeneration^[23]. 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^[23]. Recently, senescence has been reported to play important roles in the development processes and to compensate the role of apoptosis to remove unnecessary cells^[17].

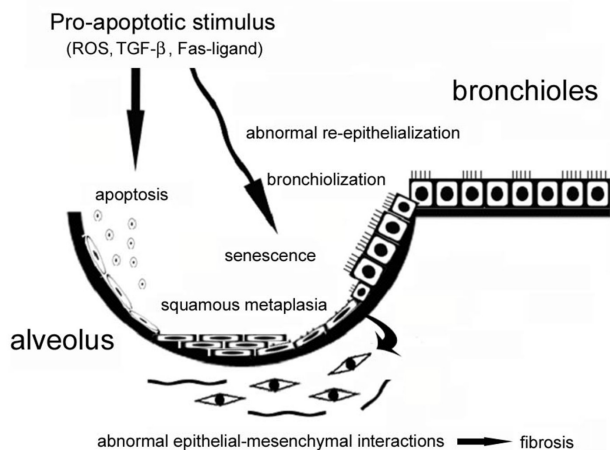


Figure 1 Apoptosis and senescence in pulmonary fibrosis. Proapoptotic factors such as ROS, TGFβ, and Fas ligand induce apoptosis in epithelial cells, while resistant cells against apoptosis or senescence migrate and proliferate to repair damaged tissues. TGF-β induces cellular senescence as well as apoptosis in bronchial epithelial cells. Regenerated epithelial cells appear cuboidal metaplasia and bronchiolization and cover remodeled tissues. IL-1β secretion as SASP by senescent epithelial cells induces myofibroblast differentiation in lung fibroblasts.

THE RELATIONSHIP BETWEEN 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^[24]. SASP may exert deleterious effects on the tissue microenvironment of neighboring cell^[24,25]. 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^[26]. 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^[26].

Indeed, autophagy diminishes with aging and accelerated aging can be attributed to reduced autophagy. Thus, autophagy activation appears to be associated with longevity^[27]. 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^[28]. 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^[29].

The role of stress-induced autophagy activation in longevity has been mainly demonstrated in the case of caloric restriction (CR)^[30]. 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)^[31]. 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^[32]. SIRT1 deacetylation of Atg proteins and transcription factors, including the FOXO family, is involved in autophagy induction^[33,34]. 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^[34]. 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^[35].

Gamerding *et al* showed that autophagy are getting more importance during the aging process, because proteasome pathway could not degrade protein aggregates in the presence of an enhanced pro-oxidant and aggregation-prone milieu characteristic of aging^[36]. Patshan *et al* investigated whether the autophagy is involved in development of premature senescence of endothelial cells^[37]. They found that pharmacological inhibition of autophagy prevented development of premature senescence but did lead to the enhanced rate of apoptosis in human umbilical vein endothelial cells^[38]. Young *et al.* showed that a subset of autophagy-related genes are up-regulated during senescence: Overexpression of one of those genes, ULK3, induces autophagy and senescence. Furthermore, inhibition of autophagy delays the senescence phenotype, including senescence-associated secretion^[39]. Goehe *et al* showed that autophagy and senescence tend to occur in parallel, and furthermore that autophagy accelerates the development of the senescent phenotype^[40]. Collectively, autophagy and cellular senescence is associated with each other in some situations, but these two important cellular processes may be interdependently involved in the pathophysiology of lung diseases.

INFECTION AND IMMUNITY

Autophagy

Autophagy has been implicated in regulation of inflammation and immunity^[41]. In the setting of bacterial and viral infections, selective autophagic degradation of intracellular pathogens for host defense is designated as xenophagy. Consistent with other selective autophagy, involvement of ubiquitination and p62 has been proposed for xenophagic recognition of intracellular microbes^[1].

In the innate immune response, there is a wide variety of close interactions between autophagy and the pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), Nod-like receptors (NLRs), and RIG-I-like receptors (RLRs). Autophagy can be triggered by TLRs during innate immune signaling^[42]. TRAF6-mediated Lys^[63] (K63)-linked ubiquitination of Beclin1 is the mechanism of autophagy activation used by TLR4, and disrupting the association between Beclin1 and Bcl2 is a proposed mechanism of autophagy activation mediated by the TLR adaptors, MyD88 and TRIF^[43]. NLRs are components of the inflammasome, an integral part of the innate immune system's response to infections and cellular stress. Inflammasome activation results in the maturation of interleukin 1β (IL-1β) and IL-18^[44]. Autophagy has been shown to negatively regulate inflammasome activation through the elimination

of dysfunctional mitochondria^[445].

In adaptive immunity, autophagy is also responsible for MHC class II antigen presentation in thymic epithelial cells (TECs), which is involved in the generation of a functional and self-tolerant CD4 T-cell repertoire^[46]. Furthermore, autophagy may have a significant role in T-cell function, including survival and proliferation, via maintaining mitochondrial clearance and ER and calcium homeostasis^[47,48].

Mycobacterium tuberculosis (Mtb) primarily targets macrophages. Stimulation of autophagy in infected macrophages significantly reduces the number and viability of intracellular Mtb^[49,50]. Vitamin D3 is a known anti-mycobacterial immunomodulator, and the active form of vitamin D induces autophagy in human monocytes via transactivation of Beclin-1 and Atg5^[51]. TLRs activation by Mtb infection induces a variety of inflammatory reactions and also induces autophagy^[52,53]. Intriguingly, TLR-induced signaling and vitamin D receptor (VDR) signaling synergistically enhance antibacterial autophagy^[54]. ROS-induced autophagy has been proposed to be a mechanism for killing of intracellular pathogens in macrophages^[53] and the "enhanced intracellular survival" gene of Mtb enhances intracellular survival of Mtb by modulating ROS-dependent autophagy^[55]. Therefore, autophagy plays a key regulatory role in clearance of pathogens and autophagy induction by appropriate stimuli can be an ambitious therapeutic option.

Cellular senescence

The change of immune system with aging is called "immunosenescence", which represents deterioration of innate and adaptive immunity resulting in impaired ability to fight against infection and to respond to vaccination, and in increase of cancer and autoimmune diseases with aging^[56,57].

In innate immunity, there are two main changes which consist of the presence of chronic inflammation shown by the increase of IL-6 and TNF- α expression, and the decreased function of specific immune effector cells^[57,58]. Chronic inflammation without obvious infection in the old is called "inflamm-aging"^[58]. Although the number of circulating neutrophils does not change with aging, the capacity to eliminate phagocytized pathogens and chemotaxis are decreased, and ROS production was increased in neutrophils in the old^[59]. The number of circulating monocytes does not decrease, but the sterilizing capacity, the expression of toll-like receptor, and cytokine production, such as IL-1 β , IFN γ , MCP-1, MIP-1 α , of macrophages are decreased in the old, which is one of the reasons that infection tends to be serious^[60]. The capacity to antigen presentation of dendritic cells to translate innate and adaptive immunity is also decreased with aging^[61]. The number of natural killer cells is increased with aging, but their capacity to cytotoxic capacity is decreased^[62]. These disabilities of killing pathogens make the old susceptible to infection and contribute to morbidity and mortality^[60].

In adaptive immunity, the number of T cells and their function are deteriorated with aging^[63]. Although the number of naive T cells is decreased in peripheral blood and tissues with aging, those of differentiated CD8, CD4 and regulatory T cells are increased^[64]. However, these T cells respond to new antigens less efficiently^[56,57]. CD8 cells accumulate in aged tissues, but do not proliferate efficiently. CD4 cells show reduced T cell receptor signaling and cytokine production after antigen binding^[65]. The lymphopoiesis of B cell is decreased, and antibodies produced by B cells have less affinity to antigens. The ability to undergo class switch recombination is impaired in the old compared with the young^[66].

Kreiling *et al* demonstrated that senescent cells accumulated

within lungs and other tissues in the old and enhanced susceptibility to bacterial infection^[67]. Although it is clear that cellular senescence with aging impairs the regulation of immune and inflammatory reactions, the effect of senescence on each kind of cells remains to be understood. In a mouse model of pneumococcus pneumonia, rapamycin prevents epithelial cell senescence and regulates the cytokine and receptor expression required for pneumococcus adherence, and prolongs the survival of mice by attenuation of lung injury caused by infection^[68]. Caloric restriction (CR) has been reported to stimulate lymphopoiesis and inhibit accumulation of senescent T cells^[69]. As shown in these results including rapamycin and CR, there are a lot of overlap in the role of autophagy and senescence in infection and immunity. Therefore, the association of autophagy and senescence is an intriguing issue in this field.

LUNG CANCER

Autophagy

Both tumor-suppressive and promoting roles have been proposed for autophagy, which may be dependent on the stage in cancer development^[70]. Autophagy suppresses generation of ROS that could damage DNA, which could contribute to cellular transformation^[71,72]. Genetic defects of autophagy gene ATG7 or heterozygous disruption of the beclin 1 with reduced autophagic activity increased the frequency of spontaneous malignancies, indicating that autophagy is a suppressive mechanism of tumorigenesis^[73]. Accumulation of p62, reflecting insufficient autophagic degradation, has been shown to be an independent prognostic factor for non-small cell lung cancer (NSCLC)^[74]. The expression of p62 promotes tumorigenesis through altered NF- κ B regulation and gene expression^[75]. Additionally, p62-mediated stabilization of Nrf2, an important transcription factor for antioxidant protein expression, may be involved in the survival of tumor cells^[76,77].

In contrast, autophagy can maintain tumor cell survival by protecting cells from oxidative stress through eliminating damaged organelles and proteins^[78,79]. Amino acids supplied by autophagy can be fundamental to survival and proliferation for established cancer cells^[2]. *In vitro* experiments demonstrated that autophagy inhibition may be a potential strategy to overcome the mechanisms of drug resistance to cancer chemotherapy and radiation in human NSCLC^[80,81]. Several clinical trials using autophagy-inhibiting agents in combination with conventional cytotoxic agents are active and recruiting as novel modalities of lung cancer treatment^[82]. Autophagy is also reported to be a fundamental requirement for maintenance of tumor stem cells^[83]. Whether autophagy has the tumor suppressive or promoting effects depends on tissues or cells. Systemic influence of autophagy induction or inhibition should be verified before clinical applications^[70].

Cellular senescence

Various stimuli also induce DNA damage which results in telomere shortening and cellular senescence, which are barrier to cell transformation and proliferation^[16,84]. When DNA damage is not repaired, damaged cells are induced to apoptosis or senescence. Therefore, apoptosis and cellular senescence are important regulatory mechanisms of carcinogenesis. Cellular senescence is dependent on two major pathways, that is, p53-p21 and p16-pRB pathways^[16,85,86]. Some deficiencies in these pathways may compromise appropriate cellular senescence and increase the susceptibility to carcinogenesis^[87]. Oncogene-induced senescence (OIS) is induced by activation of anti-oncogene such as p53, p21, and p16, which

are stimulated by cell proliferation due to oncogene activation^[88]. Persistent activation of Ras oncogene induces OIS associated with mitogen-activated protein kinase activation, and retinoblastoma protein (pRB) / p53. OIS against excessive cell proliferation is one of physiological regulatory mechanisms to inhibit carcinogenesis^[89]. Machineries responsible for autophagy and senescence can coexist in the cell treated with DNA damage agents^[90].

As well as autophagy, senescent cells do not always inhibit carcinogenesis. Rather, cellular senescence facilitates tumorigenesis^[70]. Cancer is an age-associated disease, and age is the highest risk factor for cancer. Whether cellular senescence is beneficial or deleterious may depend on the age^[18]. Deleterious effects of senescent cells is not senescent cells themselves but the accumulation of senescent cells, stem cells exhaustion, increasing damage, and the consequence of the SASP in the microenvironment. When senescent cells present for long time, SASP affects neighboring cells and induces proliferation of cancer and cancer-associated cells^[91]. In fact, many SASP factors, such as IL-6, IL-8, and VEGF, are known to promote phenotypes associated with cancer cells. It is now well known that cancer-associated senescent fibroblasts secrete factors as SASP to promote tumor development^[92,93].

BRONCHIAL ASTHMA

Autophagy

Bronchial Asthma is considered a chronic allergic inflammatory disease with Th2-type cytokine dominance, characterized by reversible airflow obstruction, airway hyper-responsiveness, and airway wall remodeling^[94]. Th1 and Th2-type immune response in viral infection is involved in the development and exacerbation of bronchial asthma^[95]. Autophagy is implicated in the pathogenic sequence in bronchial asthma in terms of regulation of immunity and viral clearance^[96]. A recent study demonstrates that single nucleotide polymorphisms (SNP) in ATG5, including a functional promoter variant, are associated with childhood asthma^[97]. Furthermore, a SNP located in intron 3 of ATG5 is associated with forced expiratory volume in 1 second (FEV1) in asthmatic patients^[98]. Although ATG5 is a crucial component of the autophagy machinery used for viral elimination, the Atg12-Atg5 conjugate has also been shown to negatively regulate the anti-viral properties of type I IFN^[97,99]. Autophagy is also modulated by both Th1 and Th2-type cytokines^[99,100]. IFN- γ , a Th1 cytokine, has been demonstrated to induce, but the Th2 cytokines, IL-4 and IL-13, inhibit starvation-induced autophagy in macrophages^[99]. Airway hyper-responsiveness is achieved by conditional Atg7 knockout in airway epithelial cells^[101]. Autophagy is also involved in regulation of ROS production and in elimination of oxidized proteins in order to minimize tissue damage^[98]. Oxidative stress is associated with airway inflammation in bronchial asthma and exhaled hydrogen peroxide (H₂O₂) and nitric oxide (NO) are associated with asthma severity^[102]. Oxidative stress is also closely associated with cellular senescence.

Senescence

Bronchial asthma is common in the old. Four to 13% of adults older than 65 years old are estimated to have bronchial asthma^[103]. Aged patients with bronchial asthma tend to be unstable and resistant to treatment, and about 90% of patients who died of bronchial asthma are the old^[104]. The number of neutrophils in bronchial walls is increased in aged patients, and inflammatory mediators such as MMP-9, elastase, and IL-8 are increased in sputum from aged patients^[105,106]. Eosinophils in bronchial walls from mouse model of

bronchial asthma are increased along with aging, in which airway wall is intensively remodeled^[107]. The serum levels of IL-17 are increased along with aging^[108]. IL-17 induces neutrophil inflammation and Th2 type eosinophilic inflammation in the airways, results in the acceleration of airway hyper-reactivity+. Chronic inflammation is a major characteristic of bronchial asthma, and is likely to induce cellular senescence in epithelial cells in patients with asthma.

In fact, the expression of senescence marker p21 and p16 are increased in bronchial epithelial cells, and the expression of type I collagen and α -SMA are increased in the airway walls from patients with bronchial asthma^[110]. Thioredoxin reduces gene expression of p21 and prevent airway remodeling in an asthma mouse model^[111]. These results suggest that cellular senescence in bronchial epithelial cells is increased along with airway remodeling^[112]. Thymic stromal lymphopoietin (TSLP) production from bronchial epithelial cells is increased in patients with bronchial asthma^[113]. TSLP plays a critical role in the inflammatory responses in asthma, through activating multiple signaling pathways, such as stat3/5, IL-1 β , and MAP kinases^[114]. Wu *et al* demonstrated that cellular senescence as shown by the expression of p21 and p16 in bronchial epithelial cells is required for TSLP-induced airway remodeling in mice, and also showed that epithelial cell senescence and airway remodeling are abrogated by stat3 inhibition^[110].

Telomere length of neutrophils in the peripheral blood is shorter in patients with asthma than that of controls^[115]. Belsky *et al.* demonstrated that asthma seemed to relate to shorter telomere length in cases with childhood onset and persistent course^[112]. They also suggested that the link between the phenotype of life-course-persistent asthma and telomere length is related to elevated systemic eosinophilic inflammation. Although the mechanisms linking the asthma and shortened telomere length are not characterized, the expression of telomerase reverse transcriptase in the airway wall is reported to be correlated with telomere shortening in circulating leukocytes from patients with bronchial asthma^[115].

It is reported that cellular senescence due to shortened telomere length is associated with the severity of bronchial asthma^[112,115,116]. Since the susceptibility to viral infection is increased along with cellular senescence of NK cells in the old^[117], the deterioration of bronchial asthma induced by infection may be easy to occur in aged patients. Cellular senescence may be involved in the effect of aging on the pathophysiology of bronchial asthma, of which mechanisms is poorly understood. Telomere shortening could be induced by oxidative stress^[118] and asthma is associated with systemic and bronchial wall inflammation and oxidative stress. Oxidative stress is associated with senescence and at least in part regulated by autophagy. Further examination is warranted to clarify the mechanisms of cellular senescence and autophagy involvement in the pathophysiology of asthma.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Autophagy

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^[2,3,7,8]. Although detailed molecular mechanisms for COPD development remain unclear, the possible participation of autophagy in the pathogenic sequence of COPD

has been intensively explored. 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^[119]. 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^[120]. 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 this COPD models. Furthermore, in cases of hyperoxia-induced apoptosis in epithelial cells, LC3B interacts with Fas, resulting in prevention of apoptosis^[121], 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^[122], indicating that autophagy activity in COPD lung may be regulated via cell type specific mechanisms.

Senescence

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^[123-125]. 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^[124,126]. 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^[8]. 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^[8]. 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 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 HBEC isolated from COPD patients compared to those from non-smokers^[8]. 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^[125]. 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 supports the notion that insufficient autophagic clearance is involved in accelerated cellular senescence in COPD^[8].

Sirtuin family belongs to Class III histone deacetylases (HDAC), and one of sirtuin family SIRT1 has extensively studied and well known as an anti-aging molecule because of SIRT1-mediated longevity by calorie restriction^[127]. SIRT1 expression is decreased in the lung tissues from patients with COPD by oxidative stress and smoking^[128]. Decreased SIRT1 expression results in the increased expression of proinflammatory cytokines due to NF- κ B activation, and also results in the acceleration of cellular senescence mediated by the decrease of anti-senescent activity through FOXO3^[129]. 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 SIRT1 knockout mice^[130]. SIRT1 activator SRT1720 suppressed emphysematous change in mice lung induced by elastase instillation and smoking inhalation^[131].

SIRT6 has been demonstrated to regulate longevity by modulating insulin-like growth factor (IGF)-I signaling^[132]. 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^[133]. 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^[134]. Decreased expression levels of SIRT6 found in lung homogenates from COPD patients supports 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 expression was significantly correlated with the decrease of FEV1%^[134]. As IGF-1 shares receptors and signaling pathways with insulin, and type 2 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 mitochondria homeostasis is prerequisite for cellular homeostasis^[135]. 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^[136]. 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 dominance of mitochondrial dynamics in COPD pathogenesis^[137]. *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^[137].

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^[138,139]. 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^[140]. 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 FEV1%. 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^[140].

IDIOPATHIC PULMONARY FIBROSIS (IPF)

Autophagy

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrosing interstitial pneumonia of unknown cause, but is influenced by a combination of genetic, epigenetic, and environmental factors^[141]. IPF is usually a lethal disease with poor prognosis, with a 3-year median survival time from the time of diagnosis and no current conventional medical intervention available to extend life span^[141]. IPF is characterized pathologically by irregular scars composed of dense collagen fibrosis alternating with areas of fibroblastic proliferation, as well as cystic remodeled airspaces lined by metaplastic epithelium, corresponding to the usual interstitial pneumonia (UIP) pattern^[142]. Advanced age is one of the most important risk factors for development of IPF, of which the prevalence is increased with aging^[141].

Autophagy has been implicated in the pathogenesis of bleomycin-induced pulmonary fibrosis in mouse, and neutralization of IL-17A attenuated bleomycin-induced pulmonary fibrosis and increased survival in epithelial cells via autophagy^[143]. A recent paper demonstrated decreased autophagy flux as measured by p62 accumulation, as well as reduced LC3-II expression levels in lung tissue homogenate from IPF patients^[144]. The authors also proposed that TGF- β -mediated autophagy inhibition in fibroblasts is responsible for myofibroblast differentiation^[144]. Additionally, increased endoplasmic reticulum (ER) stress responses have been demonstrated in metaplastic epithelial cells in IPF lung and ER stress response is known to induce autophagy, which removes the disorganized proteins to relieve cellular stress^[145]. We demonstrated that both overlying epithelial cells and fibroblasts in fibroblastic foci (FF) express both ubiquitin and p62, which appeared to reflect insufficient autophagy^[146]. FF comprised of myofibroblast accumulations is recognized to be the leading edge of fibrogenesis, and the number of FF is a potential prognostic measure^[147], further indicating the possible involvement of insufficient autophagy in IPF pathogenesis.

Interestingly, our *in vitro* experiments demonstrated that autophagy inhibition by knock down of LC3B and ATG5 was sufficient to induce myofibroblast differentiation of α -smooth muscle actin (SMA) and type I collagen expressions in lung fibroblasts even in the absence of TGF- β ^[146]. Furthermore, in contrast to recent findings^[144], TGF- β clearly induced autophagy as shown by increased LC3-II and decreased p62 levels, and autophagy inhibition further enhanced TGF- β -induced myofibroblast differentiation^[146]. Therefore,

TGF- β -induced autophagy may have a negative regulatory role in myofibroblast differentiation, which is partly consistent with a recent report in primary mouse mesangial cells^[148].

As potential mechanisms leading to insufficient autophagy in IPF lung, we speculate the involvement of chronic and latent viral infections, which have been widely implicated in IPF pathogenesis via chronic inflammation and increased apoptosis induction^[149]. Viral infections have been known to interfere with autophagy not only to prevent xenophagic degradation but also to modulate immune responses^[41]. Another possibility is aberrant activation of the PI3K-Akt-S6K1 signal pathway in response to polymerized collagen in IPF fibroblasts, which is conferred by low PTEN activity^[150]. mTOR, a negative regulator for autophagy, is a part of the PI3K-Akt-S6K1 signaling pathway, thus it is plausible that low PTEN activity may also be involved in autophagy inhibition through aberrant mTOR activation. Furthermore, IPF is recognized to be a disease of aging, and autophagy diminishes with aging^[7]. Although it is difficult to distinguish cause from consequence of disease progression, accelerated cellular senescence in epithelial cells in IPF may also be involved in the mechanism for insufficient autophagy, although that is not the case in fibroblasts.

Senescence

Telomeres protect chromosome ends from erosion and shorten with each cell division, and once a critical length is reached, cells were induced to cellular senescence or apoptosis. Telomerase restores telomere length, which consists of two essential components, telomerase reverse transcriptase (TERT) and telomerase RNA (TERC). Loss-of-function mutations in TERT have been found in 15% of familial interstitial pneumonia (FIP) and in 3% of sporadic IPF^[151,152]. These patients showed significantly shorter telomeres length in peripheral blood lymphocytes and granulocytes. In sporadic IPF, these mutations are less common; however, telomere shortening is similar to those of FIP in peripheral blood leukocytes and alveolar epithelial cells compared with those of healthy controls^[153,154]. Recently, 9 nucleotide polymorphisms including TERT gene were identified to be associated with IPF susceptibility in Japanese patients^[155].

Telomere attrition is induced by oxidative stress, smoking as well as aging, which are risk factors which have been implicated to have a role in the pathogenesis of IPF. TGF- β plays central roles in fibrogenesis and is highly expressed in lung tissues of IPF. TGF- β suppresses telomerase expression and may affect telomere shortening. Weisberg *et al* demonstrated that the decrease of telomerase expression was correlated with apoptosis of type II alveolar epithelial cells in lung tissues from patients with IPF^[156]. They suggested that low ratio of telomerase / apoptosis reduced regenerative capacity in injured lungs, which subsequently resulted in fibrosis. Zhou *et al* showed that telomerase activation ameliorated epithelial cell senescence and lung injury in bleomycin-induced pulmonary fibrosis in mice, and aged mice were susceptible to this fibrosis model^[157]. Paradoxically, Liu *et al* demonstrated that bleomycin-induced pulmonary fibrosis was attenuated in telomerase-deficient mice, and suggested that inactivation of damaged and misdirected fibroblasts by senescence due to telomerase deficiency may be beneficial to the body^[158]. Collectively, these results suggest that telomere shortening rather than telomere gene abnormality itself affects the pathophysiology of IPF. Since telomere shortening and cellular senescence is closely associated with each other, and increased cellular senescence is a major feature of aging, cellular senescence is proposed to be a part of the pathogenic sequence of IPF.

As the initiation of epithelial cell damage in IPF, proapoptotic factors such as ROS, TGF β , and Fas ligand induce apoptosis in epithelial cells, while resistant cells against apoptosis or senescence migrate and proliferate to repair damaged tissues. TGF- β induces cellular senescence as well as apoptosis in bronchial epithelial cells^[146,159]. Regenerated epithelial cells appear cuboidal metaplasia and bronchiolization and cover remodeled tissues. We have found accelerated senescence of epithelial cells, including metaplastic cells and bronchiolization, in active fibrosing lesions of IPF^[146]. Furthermore, interleukin (IL)-1 β secretion as SASP by senescent HBEC was sufficient to induce myofibroblast differentiation in lung fibroblasts, possibly playing a key role in fibrosis development^[146] (Figure 1).

Additionally, we hypothesized that epithelial cell senescence in IPF is at least partly attributed to insufficient autophagy to relieve ER stress. In the results of our study, tunicamycin (TM), an inducer of ER stress via disruption of protein glycosylation, accelerated HBEC senescence especially in the setting of insufficient autophagy^[10]. Interestingly, our *in vitro* experiments demonstrated that autophagy inhibition by knock down of LC3B and ATG5 was sufficient to induce myofibroblast differentiation indicated by α -smooth muscle actin (SMA) and type I collagen expression in lung fibroblasts even in the absence of TGF- β ^[10]. Taken together, insufficient autophagy may be an underlying mechanism of accelerated epithelial cell senescence and myofibroblast differentiation in IPF pathogenesis. Considering the insufficient autophagy and cellular senescence, we demonstrated that immunohistological staining of p21 and SA- β Gal staining were prominent in only epithelial cells covering actively fibrosing lesions, including FF. In contrast, no cellular senescence was observed in fibroblasts regardless of whether the fibrosis was mild or severe, suggesting that autophagy regulation of cellular senescence is a cell-type specific^[10].

Collectively, it is plausible that accelerated senescence of epithelial cells plays a role in IPF pathogenesis through perpetuating abnormal epithelial-mesenchymal interactions by SASP such as IL-1 β secretion. Cellular senescence and insufficient autophagy has been implicated in the pathogenesis of IPF as well as COPD. Cellular senescence in epithelial cells from patients with IPF has characteristics of premature senescence but also programmed senescence, which is also involved in embryonic organogenesis, which may be distinct from cellular senescence involved in COPD pathogenesis^[160].

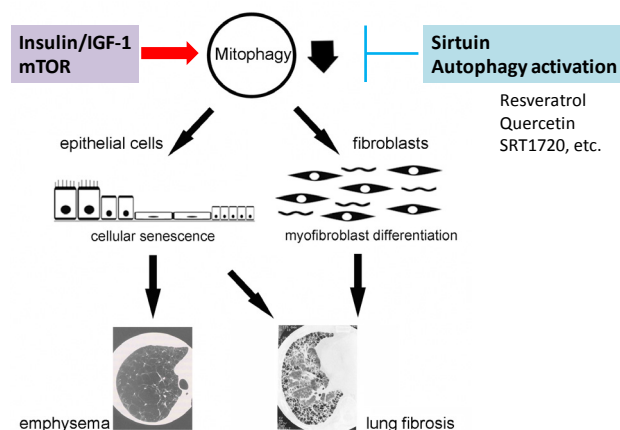


Figure 2 Therapeutic strategies targeting autophagy and senescence. The treatment strategy against cellular senescence through induction of autophagy, especially mitophagy, may be promising against lung diseases associated with aging.

CONCLUSION

An aging society has 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. Autophagy is responsible not just for simple homeostatic energy supply but also for elimination of aggregate prone proteins, damaged organelles, and intracellular microbes and also for regulation of innate and adaptive immunity as a central component of the integrated stress response. 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.

Cellular senescence is the most closely associated with aging processes, therefore, therapies targeting cellular senescence should be important strategies against various lung diseases. Cellular senescence is thought to be caused by insufficient regulatory mechanisms of homeostasis. 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 lung diseases associated with aging (Figure 2).

Recent advances in autophagy and senescence shed more light on understanding the pathogenesis of variety of pulmonary diseases and may lead to the development of new therapeutic options. For future directions, the development of proper biomarkers reflecting autophagy status and senescence is warranted to precisely evaluate autophagy and senescence status during disease progression. It is also warranted to establish novel therapeutic approaches to achieve optimal levels of autophagy status and cellular senescence.

CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

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