Autophagy in the Pathogenesis of Pulmonary Disease

Jun Araya, Hiromichi Hara and Kazuyoshi Kuwano

Abstract

Autophagy is a process of lysosomal self-degradation that helps to maintain the homeostatic balance between the synthesis, degradation and recycling of cellular proteins and organelles. Autophagy does not simply function as the machinery for supplying amino acids in response to energy demands, it is an adaptive pathway of cytoprotection against cellular stressors, including starvation, reactive oxygen species (ROS), endoplasmic reticulum (ER) stress and microbial infection. Accordingly, autophagy is considered to be the mediator of a variety of cellular processes and cell fates, including cell survival and death, cellular senescence and immune responses. Due to the organ-specific role of gas exchange, various cell types within the lungs are serially exposed to a diverse array of cellular stressors, and growing evidence has revealed the crucial involvement of autophagy in the pathogenic processes underlying pulmonary diseases. We herein review recent findings regarding the role of autophagy in cellular processes and cell fates and summarize the role that autophagy appears to play in the pathogenesis of pulmonary diseases.

Key words: autophagy, COPD, IPF, myofibroblast, senescence

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Introduction

Autophagy is a highly conserved mechanism of delivering cytoplasmic components for lysosomal degradation (1). At the cellular level, autodigestion takes place within lysosomes and proteasomes. Proteasomes, which are comprised of multisubunit structures with a narrow range of catalytic pore sizes, are involved in the clearance of ubiquitin-conjugated soluble proteins. In contrast, autophagy delivers diverse cytoplasmic components to the lysosome, including soluble proteins, aggregate-prone proteins and organelles. Three forms of distinct autophagy have been identified: chaperon (Hsc70)-mediated autophagy (CMA), microautophagy and macroautophagy (Figure). 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 via direct invagination into lysosomes. During macroautophagy, cytoplasmic components are sequestered within vesicles (autophagosomes) and delivered to the lysosome (2). Recent advances in the molecular mechanisms of autophagy have primarily focused on macroautophagy, specifically on the detection of a series of autophagy-related (ATG) genes. Hence, in general, in the literature, macroautophagy is designated as autophagy (2). Accordingly, macroautophagy is hereafter referred to as autophagy.

Among the 35 autophagy-related (Atg) proteins identified in yeast, there are core Atg proteins required for autophagosome formation that are well conserved in mammals (2). The functional associations between Atg proteins during autophagy are detailed in other reviews (3-5). Envolulfment of cytoplasmic components by the isolation membrane (phagophore) is the initial step in autophagy, followed by elongation and fusion, thus resulting in the formation of double-membranous vesicles (autophagosomes). The subsequent fusion of the autophagosome with the lysosome to form the autolysosome is absolutely required for proper degradation [Figure (6)]. Similar to the proteasomes, which is primarily responsible for supplying amino acids under a normal nutrient status, autophagy degrades 1-1.5% of total cellular protein per hour under nutrient-rich conditions in the liver (2). This basal autophagy is considered to function as part of the mechanism for cellular quality control via the proper turnover of cytoplasmic components. In addition to nutrient starvation, a wide array of cellular stressors are...
known to be strong inducers of autophagy, indicating that autophagy serves not only as a simple amino acid supply machinery in response to energy demands, but also as a central component of the integrated stress response for cytoprotection.

Initially, autophagy was proposed to be a nonselective bulk degradation system; however, recent advances have demonstrated that a variety of ubiquitinated cargo, including protein aggregates, mitochondria and microbes, are selective targets for autophagic degradation (2). Accordingly, ubiquitination is an important tag for both proteasomal degradation and 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. Due to the dynamic nature of autophagy, in which autophagosomes are formed within several minutes, it is difficult to distinguish between increased autophagy flux and impaired subsequent clearance based on electron microscopic detection of autophagosomes or examinations of the ATG expression levels. Therefore, to detect the conversion of LC3-I to LC3-II, (which is conjugated to phosphatidylethanolamine (PE) to ensure a stable association with the autophagosomal membrane), the use of protease inhibitors is generally accepted to be the standard methodology for evaluating autophagy flux. In addition, based on the findings of selective autophagic degradation, the concomitant accumulation of p62 and ubiquitinated proteins is also recognized to at least partly reflect the autophagy activity (7).

Due to the large number of physiological and aberrant intracellular components that are potential targets for autophagic degradation, the autophagy status is linked to a diverse array of cellular processes and cell fates, including energy supply, homeostatic turnover of organelles, cell survival and death, cellular senescence and immune responses (1). In terms of the pathogenic role of autophagy, excessive activation is associated with disease progression under extraphysiologic conditions (8), whereas impairment of the autophagy activity is widely implicated in the pathogenic development of a variety of human disorders. Indeed, recent in vitro and in vivo gene knockout studies have revealed that insufficient autophagy is involved in the development of lung disease (7, 9, 10). Continuous ventilation of large amounts of air with a high oxygen concentration that may contain noxious particles and harmful microbes is a fundamental function of the lungs and is required for sufficient gas exchange. Subsequently, as lung cells are serially exposed to a diverse array of cellular stressors, it is reasonable to speculate that the alleviation of autophagy-mediated cellular stress plays a key regulatory role in lung pathophysiology. We herein review the recent understanding of the participation of autophagy in cellular processes and cell fates and discuss the involvement of autophagy in the pathogenesis of the representative lung diseases included in our findings (Table).

**Autophagy and cell death (autophagic cell death and apoptosis)**

Apoptosis, type I programmed cell death, is a physiologic mechanism for cell deletion without inflammation that is necessary for the maintenance of homeostatic plasticity in

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**Table. Potential Involvement of Autophagy in Pulmonary Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cell type</th>
<th>Autophagy status</th>
<th>Mechanism</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>Epithelial cells</td>
<td>↑</td>
<td>Increased apoptosis</td>
<td>(35, 36)</td>
</tr>
<tr>
<td></td>
<td>Alveolar macrophages</td>
<td>↓</td>
<td>Decreased host defense</td>
<td>(37)</td>
</tr>
<tr>
<td></td>
<td>Epithelial cells</td>
<td>↑</td>
<td>Accelerated senescence</td>
<td>(7)</td>
</tr>
<tr>
<td>IPF</td>
<td>Epithelial cells</td>
<td>↓</td>
<td>Increased apoptosis</td>
<td>(47)</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>↑</td>
<td>Accelerated senescence</td>
<td>(10)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td></td>
<td>↑</td>
<td>Myofibroblast differentiaion</td>
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<tr>
<td></td>
<td>Tumorigenesis</td>
<td></td>
<td></td>
<td>(55)</td>
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<td></td>
<td>Tumor survival</td>
<td></td>
<td></td>
<td>(60)</td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>Epithelial cells?</td>
<td>↓</td>
<td>Increased inflammation</td>
<td>(61, 65)</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Alveolar macrophages</td>
<td>↓</td>
<td>Decreased host defence</td>
<td>(75)</td>
</tr>
</tbody>
</table>
the lungs (11). However, the cell type-specific imbalance of positive and negative regulation of apoptosis has been proposed to be a critical determinant of lung disease progression (11). Although autophagy has been postulated to be responsible for type II programmed cell death, the current understanding is that autophagic cell death is attributable simply to overwhelming autophagosome formation as a part of the stress response in which cytosol and organelles are destroyed to an unrecoverable degree (12). Autophagy is an adaptation pathway for cellular stressors, including starvation, reactive oxygen species (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 (1). Indeed, double knockout of Bax/Bak, essential components of the mitochondrial apoptotic pathway, is associated with a distinct type of cell death marked by the accumulation of autophagosomes. ATG5 knockdown ameliorates this cell death, indicating that autophagy promotes cell death in the setting of extraphysiologic apoptosis deficiency (8). 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) (13), an important constituent of the class III phosphatidylinositol (PtdIns) 3-kinase complex involved in the nucleation and assembly of the initial phagophore membrane. During receptor-mediated apoptosis, the cleavage of Atg3 proteins by caspase-8 suppresses the activation of autophagy, thereby resulting in increased cell death (14). Furthermore, irrespective of their known role in autophagy induction, several Atg proteins play diverse regulatory roles during both the apoptotic process and cell survival. Fragmented Atg5 cleaved by calpain promotes intrinsic mitochondrial apoptosis (15). Atg5 located 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 (16). LC3B has been demonstrated to regulate apoptosis by interacting with Fas and caveolin-1 (Cav-1) (17). Therefore, clarifying whether the process of autophagy is involved in the regulation of apoptosis is a prerequisite for properly interpreting experimental results in the setting of Atg manipulation.

**Autophagy and cellular senescence**

Cellular senescence has been widely implicated in disease pathogenesis in terms of both impaired cell repopulation and aberrant cytokine secretion of the senescence-associated secretory phenotype (SASP) (18). SASP can exert deleterious effects on the tissue microenvironment of neighboring cells (18, 19). Increased cellular senescence is a major feature of aging; hence, cellular senescence is widely implicated in age-associated disorders. The detailed molecular mechanism underlying the regulation of cellular senescence is complex and incompletely understood; however, one of the typical manifestations is the accumulation of damaged proteins and organelles, occasionally associated with ubiquitinated aggregation (20). Therefore, it has been proposed that a functional insufficiency in the cellular cleaning and housekeeping mechanisms of autophagy plays a pivotal role in the accumulation of deleterious cellular components and is therefore involved in the regulation of cellular senescence (20). Indeed, autophagy diminishes with age, and accelerated aging can be attributed to reduced autophagy. Therefore, the activation of autophagy appears to be associated with longevity (6).

Pathologic premature aging due to malfunctions in autophagy has been intensively examined using animal models of autophagy inhibition induced by tissue-specific knockout of ATG genes (6). These animal models of insufficient autophagy demonstrate a cellular phenotype of the progressive accumulation of ubiquitinated aggregates and disorganized mitochondria, suggesting a causal relationship between the loss of autophagy and aging-associated disease phenotypes (6). However, these phenotypic alterations have been primarily 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 the regulation of ROS of oxidative stress. Mitochondria are the primary organelle responsible for intrinsic ROS release via respiratory chain reactions, and insufficient mitophagy results in the accumulation of damaged mitochondria accompanied by increased ROS production (6, 21).

The role of stress-induced autophagy activation in longevity has been demonstrated in the case of caloric restriction (CR). CR induces autophagy via the inhibition of mammalian target of rapamycin (mTOR), an essential negative regulator of autophagy, and the activation of adenosine monophosphate-activated protein kinase (AMPK) and Sirtuin1 (SIRT1). In response to the rising AMP/ATP ratio observed during CR, AMPK induces autophagy via the phosphorylation of ULK1, a mammalian ortholog of the yeast protein kinase Atg1 (22). SIRT1 deacetylation of Atg proteins and transcription factors, including the FOXO family, is involved in the induction of autophagy (23, 24). The involvement of CR-induced autophagy in longevity is confirmed by the inhibition of autophagy, and the SIRT1-mediated longevity associated with CR is at least partly conferred by autophagy activation (24). Intriguingly, a recent paper demonstrated that SIRT1 protects against emphysema via a FOXO3-mediated reduction of premature senescence in mice; however, the involvement of autophagy was not examined (25).

**Autophagy and immune responses (inflammation and immunity)**

Autophagy has been implicated in the regulation of inflammation and immunity (26). In the setting of bacterial and viral infections, selective autophagic degradation of intracellular pathogens for host defense is designated as xeno-
phagy. Consistent with other selective autophagy processes, the involvement of ubiquitination and p62 has been proposed for the xenophagic recognition of intracellular microbes. However, the use of xenophagy against invading intracellular microbes may occasionally be encountered or even employed to proliferate microbes, as detailed in recent reviews (26, 27). There are a wide variety of close interactions between autophagy and the pattern recognition receptors (PRRs) of the innate immune response, 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. The TNF receptor associated factor (TRAF)6-mediated Lys63 (K63)-linked ubiquitination of Beclin1 is the mechanism of autophagy activation used by TLR4 (28), and the disruption of the interaction between Beclin1 and Bcl2 is a proposed mechanism of the activation of autophagy mediated by the TLR adaptors, MyD88 and TIR-domain-containing adapter-inducing interferon-β (TRIF) (26). NLRs are components of inflammasomes, 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 (29), and autophagy has been shown to negatively regulate inflammasome activation by eliminating dysfunctional mitochondria (30). Atg16L1 is an essential component of the autophagic machinery, and Atg16L1-deficient macrophages produce high amounts of the inflammatory cytokines IL-1β and IL-18 in a Crohn's disease model, further supporting the notion that autophagy is an intrinsic negative regulator of inflammasome activation (31). Autophagy is also responsible for the adaptive immunity of MHC class II antigen presentation in thymic epithelial cells (TECs), a process involved in the generation of a functional and self-tolerant CD4+ T-cell repertoire (32). Furthermore, autophagy may play a significant role in the T-cell function, including survival and proliferation, by maintaining mitochondrial clearance and ER and calcium homeostasis (33, 34). However, the involvement of the autophagic regulation of inflammation and immunity in lung pathology remains largely unknown.

**Chronic obstructive pulmonary disease and 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 (3, 5-8). Although the detailed molecular mechanisms underlying the development of COPD 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 obtained from COPD patients is augmented due to an increased LC3B-II/LC3B-I ratio and that the Egr-1-induced LC3B expression is essential for autophagy activation (35). LC3B-/- mouse experiments have confirmed the pivotal role of LC3B in the induction of epithelial cell apoptosis by cigarette smoke exposure. The proposed mechanism of LC3B-induced apoptosis is attributed to the balance of a trimolecular interaction between LC3B with Fas and Cav-1, a lipid raft protein. LC3B knockdown inhibits apoptosis by increasing Cav-1-dependent Fas sequestration, and the dissociation of Fas and LC3B from Cav-1 in response to cigarette smoke extract (CSE) exposure initiates apoptosis in epithelial cells (17). LC3B is a key component of the autophagy machinery, and the association between LC3B and Fas is an interesting observation; however, it remains unclear whether the activation of autophagy induced by the LC3B expression is crucial for the induction of apoptosis in this COPD model. Furthermore, in cases of hypoxia-induced apoptosis in epithelial cells, LC3B interacts with Fas, resulting in the prevention of apoptosis (36), suggesting that the role of the association between LC3B and Fas in the regulation of apoptosis is dependent on the stimuli or experimental conditions. Intriguingly, a decreased autophagy activity in alveolar macrophages derived from smokers has been reported in terms of impaired xenophagy. In spite of the increased level of LC3 B-II and autophagosomes in macrophages obtained from smokers, the impairment of autophagy flux has been demonstrated using protease inhibitors and by detecting the accumulation of p62 aggregates (37), indicating that the autophagy activity in COPD lungs is regulated via cell type-specific mechanisms.

COPD is assumed to be a disease of accelerated lung aging, and cellular senescence has been widely implicated in the pathogenesis of COPD, presumably due to impaired cell repopulation and the aberrant cytokine secretion observed in SASP (38-40). Autophagy plays a pivotal regulatory role in cellular senescence; hence, we attempted to elucidate the involvement of autophagy in the regulation of CSE-induced human bronchial epithelial cell (HBEC) senescence (7). CSE transiently induces the activation of autophagy followed by the accumulation of p62 and ubiquitinated proteins accompanied by an increase in HBEC senescence. The autophagy inhibition induced by 3MA, a specific inhibitor of autophagic sequestration, or LC3B and ATG5 knockdown further enhances HBEC senescence with the concomitant accumulation of p62 and ubiquitinated proteins. In contrast, the autophagy activation induced by Torin1, a mammalian target of rapamycin (mTOR) inhibitor, suppresses the accumulation of p62 and ubiquitinated proteins and inhibits HBEC senescence. In line with previous finding of increased autophagy activation in COPD epithelial cells, we observed an increase in baseline autophagy and significantly decreased autophagy induction in response to CSE exposure in HBECs isolated from COPD patients compared to that observed in HBECs isolated from nonsmokers. We speculate that the mechanism for enhanced baseline autophagy flux is attributed to increased oxidative stress, as demonstrated by our recent finding of the accumulation of carbonylated pro-
teins in HBECs obtained from COPD patients (40). Therefore, it is probable that the attenuation of autophagy flux in response to CSE exposure reflects an insufficient reserve of autophagy activation in HBECs obtained from COPD patients. The increased accumulation of p62 and ubiquitinated proteins detected in lung homogenates supports the notion that insufficient autophagic clearance is involved in the accelerated cell senescence observed in COPD (7). To further investigate the details of the role of insufficient autophagy in the regulation of HBEC senescence, we next focused on mitochondria. Parkin is an E3 ubiquitin ligase of mitochondrial proteins, and, as reported in a mouse model of Parkinson’s disease (41), we found that Parkin-mediated ubiquitination is crucial for mitophagic degradation in damaged mitochondria. Furthermore, Parkin knockdown resulted in the enhancement of HBEC senescence in response to CSE exposure, while the inhibition of autophagy induced the accumulation of damaged mitochondria accompanied by increased ROS production (Ito S. et al. in preparation for submission).

Next, to elucidate the regulatory mechanisms for autophagy in response to CSE exposure, we focused on SIRT6, a member of the sirtuin family. Sirtuin (SIRT6) has been demonstrated to regulate longevity by modulating insulin-like growth factor (IGF)-I signaling (42). IGF-I-signaling activates mTOR, and a recent paper demonstrated that IGF-I exposure is sufficient to induce cellular senescence via the inhibition of baseline autophagy (43). Intriguingly, we found that CSE-induced HBEC senescence is inhibited by SIRT6 overexpression. The histone deacetylase (HDAC) activity of SIRT6 is indispensable for the inhibition of CSE-induced HBEC senescence via the mechanism of autophagy activation, which is primarily attributed to the attenuation of IGF-Akt-mTOR signaling. The decreased expression levels of SIRT6 found in the lung homogenates obtained from COPD patients supports the hypothesis that a reduced SIRT6 expression with accompanying autophagy insufficiency is associated with the development of COPD through the enhancement of cellular senescence, especially in the setting of increased IGF signaling (44). As IGF-I shares receptors and signaling pathways with insulin, and type 2 diabetes mellitus with hyperinsulinemia is a common comorbidity in patients with COPD, these factors may be associated with the development of COPD via increased IGF/insulin signaling and autophagy inhibition, especially in cases of a decreased SIRT6 expression.

**Idiopathic pulmonary fibrosis and autophagy**

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrosing interstitial pneumonia of unknown cause, although it is influenced by a combination of genetic, epigenetic and environmental factors (45). IPF is usually a lethal disease with a poor prognosis, including a 3-year median survival time from diagnosis and no current conventional medical interventions available to extend the life span (45). IPF is characterized pathologically by irregular scars composed of dense collagen fibrosis alternating with areas of fibroblastic proliferation, as well as cystically remodeled airspaces lined by a metaplastic epithelium, corresponding to the usual interstitial pneumonia (UIP) pattern (46). Autophagy is implicated in the pathogenesis of bleomycin-induced pulmonary fibrosis in mouse models, and neutralization of IL-17A attenuates bleomycin-induced pulmonary fibrosis and increases survival in epithelial cells via autophagy (47). A recent paper demonstrated decreased autophagy flux, as measured according to p62 accumulation, as well as reduced LC3-II expression levels in lung tissue homogenates obtained from IPF patients. The authors also proposed that transforming growth factor (TGF)-β-mediated autophagy inhibition in fibroblasts is responsible for myofibroblast differentiation (48). We previously reported the accelerated senescence of epithelial cells, including metaplastic cells, in active fibrosing lesions of IPF (49). Additionally, increased ER stress responses have been demonstrated in metaplastic epithelial cells in the IPF lung, and the ER stress response is known to induce autophagy, which removes the disorganized ER to relieve cellular stress (50). Consequently, we hypothesized that the epithelial cell senescence observed in IPF is at least partly attributed to insufficient autophagy. We showed that tunicamycin (TM), an inducer of ER stress via the disruption of protein glycosylation, accelerates HBEC senescence, especially in the setting of insufficient autophagy. Next, we evaluated the autophagy status of IPF lungs using immunohistochemical evaluations. Intriguingly, both overlying epithelial cells and fibroblasts in fibroblastic foci (FF) were found to express ubiquitin and p62, which appears to reflect insufficient autophagy. FF comprised of myofibroblast accumulation are recognized to be the leading edge of fibrogenesis, and the number of FF is a potential prognostic measure (51), further indicating the possible involvement of insufficient autophagy in the pathogenesis of IPF. Immunohistological staining of p21, a senescence-associated cyclin-dependent kinase inhibitor, and senescence associated β-galactosidase (SA-β-Gal) staining are prominent only in epithelial cells covering actively fibrosing lesions, including FF. In contrast, no cellular senescence is observed in fibroblasts, regardless of whether the fibrosis is mild or severe, suggesting that the autophagic regulation of cellular senescence is cell type specific (10).

Interestingly, our in vitro experiments demonstrated that the inhibition of autophagy by the knockdown of LC3B and ATG5 is sufficient to induce myofibroblast differentiation of the β-smooth muscle actin (SMA) and type I collagen expressions in lung fibroblasts, even in the absence of TGF-β. Furthermore, in contrast to recent findings (48), 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. Therefore, TGF-β-induced autophagy may play a negative regulatory role in myofibroblast differentiation, which is partly consistent with a recent report of primary mouse mesangial cells (10, 52). Taken together, insufficient autophagy may be an underlying mechanism of accelerated epithelial
cell senescence and myofibroblast differentiation in the pathogenesis of IPF. As potential mechanisms leading to insufficient autophagy in the IPF lung, we propose the involvement of chronic and latent viral infections, which have been widely implicated in the pathogenesis of IPF via chronic inflammation and increased apoptosis induction (53). Viral infections are known to interfere with autophagy, not only to prevent xenophagic degradation, but also to modulate immune responses (26). Another possibility is the aberrant activation of the PI3K-Akt-S6K1 signal pathway in response to polymerized collagen in IPF fibroblasts, which is conferred by a low phosphatase and tensin homolog deleted from chromosome 10 (PTEN) activity (54). mTOR, a negative regulator of autophagy, functions as part of the PI3K-Akt-S6K1 signaling pathway. Therefore, it is plausible that a low PTEN activity is also involved in the inhibition of autophagy via aberrant mTOR activation. Furthermore, IPF is recognized to be a disease of aging, and inhibition of autophagy via aberrant mTOR activation. Further, a plausible involvement of chronic and latent viral infections, which have been widely implicated in the pathogenesis of IPF via chronic inflammation and increased apoptosis induction (53). Viral infections are known to interfere with autophagy, not only to prevent xenophagic degradation, but also to modulate immune responses (26). Another possibility is the aberrant activation of the PI3K-Akt-S6K1 signal pathway in response to polymerized collagen in IPF fibroblasts, which is conferred by a low phosphatase and tensin homolog deleted from chromosome 10 (PTEN) activity (54). mTOR, a negative regulator of autophagy, functions as part of the PI3K-Akt-S6K1 signaling pathway. Therefore, it is plausible that a low PTEN activity is also involved in the inhibition of autophagy via aberrant mTOR activation. Furthermore, IPF is recognized to be a disease of aging, and autophagy diminishes with age. Although it is difficult to distinguish cause from consequence in disease progression, the accelerated cellular senescence observed in epithelial cells in patients with IPF may also be involved in the mechanism underlying insufficient autophagy, although that is not the case in fibroblasts.

**Lung cancer and autophagy**

Both tumor-suppressive and -promoting roles have been proposed for autophagy, which may be dependent on the stage of cancer development (2). The heterozygous disruption of beclin 1 with reduced autophagic activity increases the frequency of spontaneous malignancies, including lung cancer, indicating that autophagy is a suppressive mechanism of tumorigenesis (55). The accumulation of p62, which reflects insufficient autophagic degradation, has been shown to be an independent prognostic factor for non-small cell lung cancer (NSCLC) (56), and the p62 expression promotes tumorigenesis via mechanisms of altered NF-kB regulation and gene expression (57). Additionally, the p62-mediated stabilization of Nrf2, an important transcription factor for the antioxidant protein expression, may be involved in the survival of tumor cells (58, 59). On the other hand, the amino acids supplied by autophagy are fundamental to the survival and proliferation of established cancer cells (2). In vitro experiments have demonstrated that autophagy inhibition is a potential strategy to overcome the mechanisms of drug resistance to cancer chemotherapy in human NSCLC (60), and several clinical trials using autophagy-inhibiting agents in combination with conventional cytotoxic agents are active and recruiting patients to assess novel modalities of lung cancer treatment. However, both the tumor-suppressive and -promoting sides of autophagy activation and the systemic influence of autophagy inhibition must be taken into account for clinical application.

**Bronchial asthma and autophagy**

Bronchial asthma is considered to be a chronic allergic inflammatory disease with Th2-type cytokine dominance. In the development of bronchial asthma, the initial Th1-type immune response to viral infection is recognized to be a prerequisite for progression to the subsequent dominant Th2-type response. Therefore, it is not surprising that autophagy is implicated in the pathogenic sequence of bronchial asthma in terms of the regulation of immunity and viral clearance. A recent study demonstrated that single nucleotide polymorphisms (SNPs) in ATG5, including a functional promoter variant, are associated with childhood asthma (61). Another group also reported that an SNP located in intron 3 of ATG5 is associated with the prebronchodilator forced expiratory volume in 1 second (FEV1) in asthmatic patients (62). Although ATG5 is a crucial component of the autophagy machinery used for viral elimination, the Atg12-Atg5 conjugate has been shown to negatively regulate the antiviral properties of type I interferon (IFN) (61, 63). Therefore, the exact role of ATG3 gene polymorphisms in the pathology of bronchial asthma remains unclear. In addition to the regulatory role of autophagy in immune responses, autophagy is modulated by both Th1 and Th2-type cytokines (63, 64). IFN-γ, a Th1 cytokine, has been demonstrated to induce autophagy, while the Th2 cytokines IL-4 and IL-13 inhibit starvation-induced autophagy in macrophages (63). However, the role of Th2-type cytokine-mediated autophagy inhibition in bronchial asthma remains unclear. Airway hyperresponsiveness to cholinergic stimuli, an underlying pathology for the development of bronchoconstriction in patients with bronchial asthma, is achieved by conditional Atg7 knockout in airway epithelial cells (65). Epithelial cell damage resulting from autophagy inhibition induced by Atg7 deletion is proposed to be a potential mechanism for this hyperresponsiveness. Autophagy is also involved in the regulation of ROS production and the elimination of oxidized proteins in order to minimize tissue damage (62). Oxidative stress is associated with airway inflammation in patients with bronchial asthma, and the levels of exhaled hydrogen peroxide (H₂O₂) and nitric oxide (NO) are associated with the severity of asthma (66). Accordingly, further examinations are warranted to clarify the clinical implications; however, there are several plausible mechanisms underlying the involvement of autophagy in the pathogenesis and severity of bronchial asthma, including the modulation of immune responses, viral clearance and ROS regulation.

**Tuberculosis and autophagy**

Tuberculosis, a representative pulmonary infectious disease, continues to be a major cause of mortality worldwide. The causative agent, Mycobacterium tuberculosis (Mtb), primarily targets macrophages, and autophagic elimination is crucial for host defensive mechanisms during Mtb infection. Recent in vitro experiments have shown that the stimulation of autophagy in infected macrophages, either by physiological or pharmacological stimuli, significantly reduces the number and viability of intracellular Mtb microbes (67). Extracellular ATP binding to P2X₇, a plasma membrane recep-
tor, induces Ca\(^{2+}\)-dependent autophagy, which is responsible for Mtb elimination in human macrophage-like THP-1 cells (68). Vitamin D3 is a known antimycobacterial immunomodulator, and 1,25-dihydroxyvitamin D3 (1,25D3), the active form of vitamin D, induces autophagy in human monocytes via the cathelicidin-mediated transactivation of Beclin-1 and Atg5 (69). Regarding TLRs, TLR2 is responsible for the recognition of mycobacterial cell wall antigens, while TLR4 recognizes heat shock proteins and TLR9 is activated by mycobacterial DNA (70). The activation TLRs by Mtb infection induces a variety of inflammatory reactions in addition to autophagy (71). Intriguingly, TLR-induced signaling and vitamin D receptor (VDR) signaling synergistically enhance antibacterial autophagy (72). ROS-induced autophagy has been proposed to be a mechanism for killing intracellular pathogens in macrophages (71), and the “enhanced intracellular survival” (eis) gene of Mtb enhances the intracellular survival of Mtb by modulating ROS-dependent autophagy (73). Interferon gamma (INF-\(\gamma\)) has also been shown to induce autophagy, and autophagy activation by INF-\(\gamma\) is indispensable for the inhibition of Mtb survival in macrophage cell lines (74). Mice lacking INF-\(\gamma\) inducible immunity-related GTPase (IRGM1), which is required for autophagy induction by INF-\(\gamma\), fail to control Mtb replication. Recently, an Mtb infection model of Atg5\(^{lox/lox}\) LysM-Cre\(^{+}\) mice with the selective genetic deletion of Atg5 in myeloid cells, displayed an increased bacillary burden, further confirming the in vivo role of autophagy in controlling Mtb infection (75). Genome-wide siRNA screening to identify host factors associated with the intracellular survival of Mtb in THP-1 cells selected a number of candidates that collectively interfere with autophagy-inducing pathways at multiple points (76). Furthermore, a variety of gene polymorphisms of P-X, VDR, IRGM1 and TLRs are associated with either susceptibility or resistance to Mtb infection, yet the clinical implications of autophagy in patients with tuberculosis remain to be determined (77). However, it is likely that autophagy plays a key regulatory role in the clearance of Mtb to prevent tuberculosis, and the induction of autophagy by appropriate stimuli is an ambitious therapeutic option that appears to be promising, especially in the setting of drug resistance.

**Summary**

Autophagy is responsible not only for supplying simple homeostatic energy, but also for eliminating aggregate prone proteins, damaged organelles and intracellular microbes and regulating both innate and adaptive immunity as a central component of the integrated stress response. Recent advances in autophagy have shed more light on the pathogenesis of a variety of pulmonary diseases and may lead to the development of new therapeutic options. However, autophagy is a dynamic process that can rapidly change its status, influenced by both the disease activity and environmental stressors. Additionally, the regulatory role of autophagy is dependent on the stage of disease development, and the pathogenic involvement may differ in a cell type-specific manner. Furthermore, both insufficient autophagy and prolonged or excessive autophagy are potentially harmful. Therefore, for future directions, the development of proper biomarkers reflecting the autophagy status is warranted in order to precisely evaluate the autophagy status during disease progression and establish novel therapeutic approaches to achieve optimal levels of autophagy activation.

**The authors state that they have no Conflict of Interest (COI).**

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