Autophagy in Pulmonary Diseases

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Abstract

The pathogenesis of pulmonary diseases is often complex and characterized by multiple cellular events, including inflammation, cell death, and cell proliferation. The mechanisms by which these events are regulated in pulmonary diseases remain poorly understood. Autophagy is an essential process for cellular homeostasis and stress adaptation in eukaryotic cells. This highly conserved cellular process involves the sequestration of cytoplasmic components in double-membrane autophagosomes, which are

delivered to lysosomes for degradation. The critical roles of autophagy have been demonstrated in a wide range of pathophysiological conditions. Emerging studies have identified that autophagy plays important roles in the pathogenesis of various lung diseases. In addition, autophagy has been shown to selectively degrade subcellular targets, including proteins, organelles, and pathogens. Here, we highlight the recent advances in the molecular regulation and function of autophagy in lung diseases.

Keywords: autophagy; pulmonary diseases; mitochondria

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Despite recent progress in patient care and interventions, many pulmonary diseases still lack effective treatments and remain incurable diseases. Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States (1), and lung cancer is by far the leading

cause of cancer death among both men and women (2). Thus, there is a need to develop new therapeutic targets for such pulmonary diseases. Macroautophagy (hereafter referred to as autophagy) is a cellular self-degradation process that facilitates the lysosomal breakdown of intracellular material and organelles sequestered within autophagosomes (3–5). This degradation and recycling process is essential for cellular homeostasis and stress adaptation and is well conserved among most of eukaryotic life. During autophagy, cytosolic “cargos” (e.g., proteins, lipids, and organelles) are delivered into double-membrane vesicles, termed autophagosomes, and subsequently fuse with lysosomes (Figure 1) (3–5). Autophagy cargos sequestered in lysosomes are digested by lysosomal hydrolases to their basic components (e.g., amino acids and fatty acids), which are recycled for macromolecular synthesis and energy production for cell survival. In addition, autophagy exerts cytoprotective functions by removing

potentially “harmful” cytosolic substances, such as protein aggregates or damaged organelles (e.g., mitochondria) (3, 4, 6). A failure to clear deleterious cytosolic substrates, such as damaged mitochondria, may cause cellular dysfunctions that lead to pathological conditions (3–5). Autophagy is highly inducible by environmental stressors, and it thereby constitutes an important part of the mammalian stress and adaptive responses.

Since the autophagosome structure was observed in mammalian cells in the 1950s (7), the formation of autophagy has been reported in various cell types and tissues (8). Initial studies describing the molecular mechanisms and functions of autophagy were mainly from genetic screens including identifying a series of autophagy-related genes (*Atg*) in yeast, such as *Saccharomyces cerevisiae* (7). To date, a number of studies suggest that autophagy is regulated in various pathophysiological conditions and functionally involved in the pathogenesis and development of diseases including

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Figure 1. Schema of the autophagy pathway. The autophagy pathway proceeds through several phases, including initiation (formation of a preautophagosomal structure leading to an isolation membrane, or phagophore), vesicle elongation, autophagosome maturation and sequestration of cytosolic cargo, and autophagosome–lysosome fusion. In the final stage, lysosomal acid hydrolases degrade autophagosomal contents, which are released for metabolic recycling.

cancer, neurodegenerative disorders, and metabolic and infectious diseases (3–5, 9). In addition, recent studies suggest that autophagy can selectively degrade subcellular proteins, organelles, and bacteria in processes termed “selective autophagy” (3, 4, 10). Since our review of autophagy in the *Journal* in 2011 (11), a number of advances have been made, especially in our improved understanding of the functional roles of autophagy in experimental and human lung diseases. In this review, we aim to summarize the current understanding of the regulation and function of autophagy, with an emphasis on the importance of selective autophagy in the pathogenesis of lung diseases and its therapeutic targets.

Molecular Regulation of Autophagy

Environmental cues activate autophagy through regulatory factors that initiate autophagy machinery, which consists of homologs of similar autophagy-related (Atg) proteins originally identified in yeast (7). The autophagy process can be divided into six steps (Figures 1 and 2): (1) activation of molecular signaling pathways (by nutrient signals), (2) isolation of membrane as an initiation step, (3) vesicle elongation, (4) cargo loading and maturation (autophagosome formation), (5) fusion with lysosome (autolysosome formation), and (6) degradation. Autophagy is negatively regulated by nutrient and growth factor–related signals through the activation of the mammalian target of rapamycin (mTOR) (Figure 2) (3–5, 7). In contrast, nutritional starvation activates

autophagy through the inhibition of the mTOR pathway (Figure 2). The autophagy pathway is also promoted by the activation of the 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) in response to energy depletion (Figure 2). Along with these molecular regulators and signals, Beclin 1 participates in autophagosomal nucleation and formation (Figure 2). The elongation and maturation of the autophagosome requires two ubiquitin-like conjugation systems: the ATG5-ATG12-ATG16L1 and the microtubule-associated protein-1 light chain-3 (LC3) conjugation systems (Figure 2). In the final stage, autophagosomal contents are degraded by lysosomal acid hydrolases, and the contents of the autolysosome are released for metabolic recycling (Figure 1).

Measurement of Autophagy and Mitophagy

The growing interest in the field of autophagy has led to a number of technical and scientific advances *in vitro* and *in vivo*. We provide an updated summary of the measurement of autophagy and its activity in the online supplement (Table E1).

Function of Autophagy

Autophagy acts as a survival mechanism under conditions of various environmental stresses to maintain cellular integrity by regenerating metabolic precursors and clearing subcellular debris. Previously,

autophagy was believed to be a process whereby subcellular compartments are recycled randomly through nonspecific encapsulation of cytosolic materials. However, recent studies have demonstrated that autophagy selectively recognizes and captures substrates, a process that is regulated by cargo-specific factors (selective autophagy) (Figure 3) (3, 5, 10). Furthermore, this specialized autophagy has also been shown to regulate various pathophysiological conditions (3, 4). The modification of subcellular targets by ubiquitination is a critical step for identification of selective autophagy substrates (3, 5, 10). Selective autophagy contributes to the turnover of cellular organelles (e.g., mitochondria through the process of “mitophagy”) (Figure 4) and the clearance of polyubiquitinated protein aggregates (i.e., aggrephagy) (3–5, 10). Dysfunctional mitochondria or protein aggregates can be accumulated during stress, aging, and diseases, which may lead to further increase of oxidative stress, inflammation, and cell death. Therefore, defective selective autophagy may exacerbate pathological conditions or severity of diseases. Interestingly, autophagy is also implicated as a regulator of lipid metabolism (i.e., lipophagy) and bronchial cilia (i.e., ciliophagy) (4).

Although autophagy machinery acts as a cell survival mechanism to prevent cell death, interactions of autophagy- and apoptosis-related molecules such as Beclin 1 and BCL-2 (12), or LC3B and Fas (13), suggest complex cross-talk between cell survival and death. A recent study suggests implication of selective autophagy (mitophagy) on necroptosis, a programmed

Figure 2. Molecular mechanism of autophagosome formation. Nutrient signals modulate mammalian target of rapamycin (mTOR) pathways. In response to insulin or other growth factors, class I phosphatidylinositol-3-kinase (PI3K)–AKT activates mTOR, which acts as a negative regulator of autophagy. AKT may also negatively regulate autophagy by phosphorylating Beclin 1. Cellular energy depletion (e.g., decrease of intracellular ATP/adenosine monophosphate [AMP] ratio) can activate AMP-activated protein kinase (AMPK). Activated AMPK negatively regulates mTOR and consequently activates UNC-51-like kinase 1 (ULK1), thereby acting as a positive regulator of autophagy in response to energy depletion. The mTOR protein resides in mTOR signaling complex 1 (mTORC1), consisting of the regulatory-associated protein of mTOR (Raptor), G protein β subunit-like protein (G β L), and proline-rich Akt substrate of 40 kD (PRAS40), which in turn negatively regulates the mTOR substrate complex consisting of ULK1, autophagy-related (ATG)13, ATG101, and FIP200. Under conditions of nutrient depletion, mTORC1 activity is inhibited and therefore promotes ULK1 activity, leading to autophagy activation. Autophagy is also activated by the Beclin 1–interacting complex, consisting of Beclin 1, class III phosphatidylinositol-3-kinase (PIK3C3, or VPS34), and ATG14L. This Beclin 1 complex generates phosphatidylinositol-3-phosphate (PI3P), which promotes autophagosomal membrane nucleation. B-cell lymphoma 2 (BCL-2) family proteins interact with Beclin 1 to inhibit autophagy. Autophagosomal elongation requires two ubiquitin-like conjugation systems. The ubiquitin-like protein ATG12 is conjugated to ATG5 by ATG7 and ATG10 enzymes. The ATG5–ATG12 complex further forms a complex with ATG16L1, which promotes elongation of the autophagy membrane. Another conjugation system requires microtubule-associated protein 1 light chain 3 (LC3). LC3 is modified with the cellular lipid phosphatidylethanolamine (PE). The precursor form of LC3 is cleaved by the protease ATG4B to generate the LC3-I. ATG7 and ATG3 participate in the conjugation of PE with LC3-I (free form) for conversion to LC3-II (PE-conjugated form). In mammals, this conversion of LC3-I to LC3-II is a key regulatory step in autophagosome formation. The conversion of LC3-I (unconjugated cytosolic form) to LC3-II (autophagosomal membrane-associated PE-conjugated form) is regarded as an indicator of autophagosome formation. FIP200 = FAK family kinase–interacting protein of 200 kD.

necrosis (14), which is regulated by several key molecules, including mixed lineage kinase domain-like protein (MLKL) and receptor interacting serine/threonine-protein kinase 3 (RIPK3), and implicated in various human diseases (15).

Autophagy also regulates various immune responses during infection. Autophagy contributes to the host defense mechanism by providing intracellular lysosomal degradation of invading pathogens, termed “xenophagy” (Figure 3) (9, 16, 17). In addition to its direct role in pathogen clearance, autophagy also enhances host defense by increasing

immune recognition of infected cells via the generation of antigenic bacterial peptides (18). Since Beclin 1 was shown to be protective against α virus encephalitis in 1998 (19), many studies have demonstrated the protective role of autophagy against invading pathogens (16, 17). Microbes that can be eliminated by autophagy include bacteria (e.g., group A *Streptococcus*, *Mycobacterium tuberculosis* (Mtb), *Shigella flexneri*, *Salmonella enterica*, *Listeria monocytogenes*, and *Klebsiella pneumoniae*), viruses (e.g., herpes simplex virus type 1 and chikungunya virus), and parasites such as

Toxoplasma gondii (16, 17). However, emerging studies suggest that the functions of autophagy on host defense differ among microbes. Some microbes, such as hepatitis C virus or HIV-1, can adapt to autophagy machinery and evade or use autophagy machinery to promote survival or replication (16, 17).

To avoid excess inflammation during infection, selective autophagy also participates in the regulation of immune responses. Invasion of microbes can cause oxidative stress and dysregulated mitochondrial function. Mitophagy controls innate immune responses by preserving

Figure 3. Subtypes of selective autophagy. Selective autophagy refers to the specific targeting of cellular subcomponents for autophagy degradation. The turnover of mitochondria through selective autophagy pathways is termed “mitophagy.” The capacity of autophagy to clear intracellular pathogens, such as bacteria, viruses, and parasites, is collectively referred to as “xenophagy.” “Aggrephagy” refers to the selective autophagy degradation of cytosolic protein aggregates. The specific degradation of airway epithelial cilia components is regulated by autophagy (“ciliophagy”). Autophagy also regulates intracellular lipid stores, cellular levels of free lipids such as fatty acids, and energy homeostasis (“lipophagy”). Defect of distinctive selective autophagy may cause accumulation of harmful cytosolic bulks (e.g., intracellular pathogens, dysfunctional mitochondria, and protein aggregates) or metabolic deregulation, resulting in excessive immune responses, cell death, or pathogen growth. In contrast, selective autophagy, such as mitophagy, may contribute to pro-cell survival in certain oxidative stress conditions (e.g., exposure to cigarette smoke).

mitochondrial integrity. Mitophagy deficiency may lead to the accumulation of dysfunctional mitochondria with increased mitochondrial reactive oxygen species (mtROS) generation and the leakage of (oxidized) mitochondrial DNA (mtDNA) into cytosol (Figure 5A) (20–22). Consequently, this accumulation of damaged mitochondria due to defective mitophagy promotes immune responses, in particular the inflammasome (4, 21, 22). Inflammasome is a multiprotein complex to promote caspase-1-mediated secretion of proinflammatory cytokines IL-1 β and IL-18 and pyroptosis (macrophage cell death), which is implicated in a wide range of human diseases (23). Autophagy (mitophagy) machinery has been shown as a critical negative regulatory mechanisms of the inflammasome. The functions of autophagy on type I IFN signaling pathways in viral infection models are

also well reported (Figure 5B and 5C). Autophagy activation negatively regulates innate antiviral immune responses by inhibiting interaction of retinoic acid-inducible gene-I-like receptors and mitochondrial antiviral signaling protein (also known as IPS-1, VISA, and CARDIF) (Figure 5B) (24). ATG9 inhibits the activation of simulator of IFN gene (STING) by inhibiting assembly of STING and TANK-binding kinase I, resulting in the suppression of type I IFN production (Figure 5C) (25). Beclin 1 suppresses type I IFN response by directly binding to cyclic GMP-AMP synthase in response to viral DNA (26).

Autophagy is also critically involved in the adaptive immune system by affecting the functions of T cells, memory B cells, and plasma cells (9, 16, 17). Autophagy regulates the balance between myeloid and lymphoid progenitors and their

differentiation (9). Furthermore, autophagy is involved with major histocompatibility complex class II antigen presentation for both intracellular and extracellular antigens and enhances antimicrobial host defense (9, 17). For example, autophagy delivers cytosolic proteins to the lumen of antigen-processing compartments and promotes major histocompatibility complex class II antigen presentation in extracellular space (9, 17). Autophagy can also contribute to the maintenance of memory B cells and regulate the secretion of immunoglobulins (9, 27, 28).

Thus, autophagy is implicated a wide range of cellular functions and physiology. Pathological relevance of autophagy and genetic mutation of autophagy in human diseases is described in the online supplement.

Autophagy in Pulmonary Diseases

As diverse pathophysiological roles of autophagy have been revealed, a number of studies exploring the roles of autophagy in lung disease also have been reported. Figure 6 and Table E2 show recent studies showing the regulation and functions of autophagy in the field of lung. In this section, we discuss representative pulmonary diseases in which autophagy (particularly selective autophagy) is implicated in experimental pulmonary disease models and patients with pulmonary diseases.

COPD

Autophagy in COPD. In 2000, the potential implication of autophagy in pulmonary diseases was realized when autophagic vacuoles were observed in a liver biopsy specimen from a patient with alpha-1 antitrypsin deficiency, a mutation associated with the development of emphysema (29). Although emphysema can be developed even without a history of significant smoking in patients with alpha-1 antitrypsin deficiency, this observation enabled physicians and scientists to hypothesize that autophagy is involved in the pathogenesis of COPD. Chen and colleagues reported that the expression of LC3B-II, a marker of autophagosome formation, and key autophagy-associated proteins such as ATG4, ATG5–ATG12, and

Figure 4. Molecular mechanism of mitophagy. In healthy mitochondria, PARL cleaves PTEN-induced putative kinase 1 (PINK1). On mitochondrial stress, activated PINK1 becomes stabilized on the outer mitochondrial membrane, phosphorylates serine 65 (Ser65) of ubiquitin, and activates cytosolic Parkin, which assembles polyubiquitin chains on the outer mitochondrial membrane. Subsequently, optineurin (OPTN) and nuclear dot protein 52 (NDP52) are recruited to phosphorylated ubiquitin on mitochondria (17, 18). PINK1 also recruits OPTN and NDP52, which recruit ULK1, DFCEP1, WIPI1, and LC3 to generate autophagosome-initiating autophagy machinery. DFCEP1 = double FYE-containing protein 1; LC3 = microtubule-associated protein light chain 3; PARL = presenilins-associated rhomboid-like protein; PTEN = phosphatase and tensin homolog; ULK1 = UNC-51-like kinase 1; WIPI1 = WD-repeat domain phosphoinositide-interacting 1.

ATG7 was increased in COPD lung relative to control lung (30). Electron microscopic analysis revealed increased autophagosome formation in lung tissues from patients with COPD compared with control tissues (30).

In experimental models of COPD, autophagy proteins and autophagosome formation are also increased in the lungs of mice exposed to environmental cigarette smoke (CS), which induces air space enlargement in the lung. Importantly, LC3B-deficient mice are resistant to CS-induced air space enlargement (13), suggesting the implication of autophagy in the pathogenesis of emphysema (loss of alveolar surface area). Subchronic CS exposure for 3 weeks disrupts mucociliary clearance in the airways

of mice, indicating the pathogenesis of bronchitis associated with COPD (31). Autophagy protein-depleted mice are also resistant to mucociliary clearance disruption in the airways after CS exposure (31). These studies indicate that the autophagy pathway contributes to the development of COPD.

Implication of selective autophagy in COPD. The mechanisms underlying the pathogenesis of COPD remain incompletely understood but implicate aberrant cellular responses in bronchial cells, alveolar epithelial cell, and immune cells (e.g., alveolar macrophages) in response to CS (32–34). Although promoting epithelial cell death by autophagy in the context of CS was shown as a potential mechanism (13, 30),

it has remained unclear how and which selective autophagy contributes to the development of COPD. The implication of mitochondria in COPD has been reported (35, 36); therefore, it is plausible that selective autophagy machinery can target dysfunctional mitochondria in COPD. Indeed, the subsequent study demonstrated that a key mitophagy protein, phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1), is increased in the lungs of patients with COPD (14). Genetic deficiency of PINK1 and pharmacological inhibition of mitophagy by Mdivi-1 ameliorates mitochondrial dysfunction, air space enlargement, and mucociliary clearance disruption in mice during CS exposure (14). Mechanistically, mitophagy contributes to the development of COPD by promoting necroptosis, a programmed form of necrotic cell death (14). Although the precise mechanism by which mitophagy regulates necroptosis in response to CS remains unclear, these results suggest the important roles of mitophagy in determining cell death modes.

Selective autophagy also contributes to mucociliary clearance disruption during CS exposure. CS exposure promotes shortening of respiratory epithelial cell cilia and increases histone deacetylase 6 (HDAC6) in tracheal epithelial cells. HDAC6, a critical regulator of primary cilia resorption, also regulates autophagy at the step of autophagosome-lysosome fusion (31). CS-induced cilia shortening is suppressed in autophagy protein-depleted mice and HDAC6-deficient mice (31). Thus, autophagy also targets bronchial cilia for degradation (i.e., ciliophagy) mediated by HDAC6 in COPD models (37).

In contrast to these selective autophagy modes (mitophagy and ciliophagy), alveolar macrophages from smokers display loss of autophagy activity and defective delivery of bacteria to lysosomes (38). The digestion of intracellular microbes through autophagy machinery (xenophagy) is an important host defense system in alveolar air space. The impairment of this selective autophagy in alveolar macrophages by CS may be a possible cause of the recurrent airway infections in patients with COPD. Thus, multiple selective autophagy pathways are involved in COPD models.

These studies now raise questions regarding the regulation and function of

Figure 5. Immunological functions of autophagy. (A) Autophagy can negatively regulate inflammasome-mediated maturation and secretion of proinflammatory cytokines (e.g., IL-1 β and IL-18) in response to pathogens or pathogen-associated molecules. During inflammatory stimuli, mitochondria are damaged and increase mitochondrial reactive oxygen species (mtROS) generation and translocation of mitochondrial DNA (mtDNA) to cytosol, which promotes NLRP3-mediated inflammasome activation. Absence of autophagy increases accumulation of dysfunctional mitochondria and enhances inflammasome activation. (B) ssRNA viruses are recognized by the members of the retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) in the cytosol. ATG5–ATG12 conjugates block RLR signaling by direct C-terminal caspase-recruitment domain-mediated association with RIG-I and MAVS, which reside in mitochondria. ATG5 deficiency enhances RLR signaling and increased IFN secretion, accompanied by increased mtROS generation. (C) Autophagy contributes to the down-regulation of IFN-mediated immune response. Cytosolic dsDNA, such as cytosolic mtDNA or viral DNA, activates cyclic GMP–AMP (cGAMP) synthase to form 2'3'-cGAMP, which activates stimulator of IFN genes (STING) through a series of structural changes. Activated STING then recruits TBK1 to phosphorylate IFN regulatory factor 3 (IRF3), resulting in the production of type I IFNs and other cytokines. ATG9 can suppress activation of STING, therefore regulating IRF3-mediated proinflammatory cytokine production. ATG = autophagy protein; DAMP = damage-associated molecular pattern; dsDNA = double-stranded DNA; MAVS = mitochondrial antiviral-signaling protein; NLRP3 = NLR family, pyrin domain-containing 3; PAMP = pathogen-associated molecular pattern; ssRNA = single-stranded RNA; TBK1 = TANK-binding kinase-1.

autophagy in COPD. First, given that a number of studies showed protective effects of autophagy in various stress models, why does autophagy (particularly mitophagy and ciliophagy) display deleterious effects in COPD models? One hypothesis is that continual activation of autophagy by chronic CS exposure may exceed the beneficial aspect of autophagy-mediated cell death (e.g., actively removing dysfunctional epithelial cells), which may lead to disruption of alveolar structure. Second, how can CS differentially regulate selective autophagy between epithelial cells and macrophages? It remains unclear how autophagy machineries are differentially regulated among cell types;

however, this unique regulation of autophagy may be partly due to cell-specific metabolic regulation (39). Finally, it is also important to investigate cross-talk of autophagy among different organs or tissues. COPD is known to be associated with various complications, such as anemia, osteoporosis, and muscle dysfunction. For example, in the vastus lateralis, LC3B protein lipidation is increased in patients with COPD and inversely correlates with thigh cross-sectional area, FEV₁, and FEV₁/FVC ratio (40). Although further studies will be needed to elucidate the precise roles of autophagy in COPD, these studies suggest that autophagy is not just a cellular recycling system, but it exerts

regulations and functions among different types of cells in COPD.

Cystic Fibrosis

Selective autophagy in cystic fibrosis. Cystic fibrosis (CF) is a fatal autosomal recessive disease, which is caused by mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (41–43). CF is characterized by abnormally viscous mucous, which obstructs organ passages, resulting in recurrent pulmonary infections. The most common CFTR mutation is a deletion of phenylalanine at position 508 (CFTR^{F508del})

Figure 6. Roles of autophagy in pulmonary diseases. Autophagy may prevent or promote the development or progression of pulmonary diseases by exerting its diverse functions. These functions include the regulation of cell death (or survival), innate and adaptive immune responses, and cell proliferation. Specialized functions of autophagy (selective autophagy, such as mitophagy or xenophagy) may more directly contribute to the regulation of pathogenesis in pulmonary diseases. COPD = chronic obstructive pulmonary disease; IPF = idiopathic pulmonary fibrosis; PAH = pulmonary arterial hypertension; SMA = smooth muscle actin; Th1 = T helper type 1.

in the CFTR gene (41, 43). Knockdown of CFTR or overexpression of CFTR^{F508del} results in increased ROS production and tissue transglutaminase, which leads to inactivation of key autophagy molecule Beclin 1 (44). Cells with CFTR^{F508del} display accumulated polyubiquitinated proteins, decreased clearance of aggresome, and defective autophagy (44, 45). Importantly, restoration of autophagy by overexpression of Beclin 1 ameliorates inflammatory responses (44), suggesting a critical role of removal of protein aggregate by autophagy machinery (i.e., aggrephagy). Similarly, mice bearing the F508del-CFTR mutation whose phenotypes include body weight loss and short lifespan also display low levels of Beclin 1 expression (46). In addition to the significance of aggrephagy, impairment of xenophagy in CF may be responsible for the recurrence of infection. Mice harboring the F508 mutation display exacerbated acute lung injury in response to infection with *Burkholderia cenocepacia* (47). However,

restoration of autophagy by rapamycin reduces the bacterial number and suppresses lung inflammation and injury (47).

Significance of selective autophagy in patients with CF. Selective autophagy may also affect intervention for patients with CF. Long-term use of azithromycin has been shown to improve clinical outcomes in patients with pulmonary diseases such as CF and COPD (48, 49), possibly due to its antiinflammatory properties (50). However, several studies report a synchronous increase of mycobacterial infection in patients with CF, predominantly with the highly pathogenic nontuberculous mycobacteria *Mycobacterium abscessus* (51, 52). This paradoxical consequence of azithromycin treatment in patients with CF may be due to the inhibitory effect of azithromycin on lysosomal acidification, thereby impairing autophagic and phagosomal degradation. As a result, azithromycin may inhibit intracellular killing of mycobacteria within macrophages

of the patients (i.e., impairment of xenophagy) (53). Therefore, the increased infection rates with nontuberculous mycobacteria seen in patients with CF using azithromycin may be due to decreased xenophagy induced by the drug in addition to dysregulated autophagy in patients with CF. These studies highlight the significance of selective autophagy activity in CF.

Therapies that restore autophagy function may offer an additional therapeutic potential in the treatment of CF. Interestingly, oral treatment of cysteamine, a reduced form of cystamine and an approved drug for the treatment of cystinosis (54), restores Beclin 1 expression and improves the lifespan of F508del-CFTR mutant mice (54). In fact, cysteamine is known to induce autophagy, and therefore it may be valuable to study cytoprotective mechanisms of cysteamine as a potential therapeutic agent for CF through restoration of autophagy (54, 55).

Mtb Infection

Autophagy promotes Mtb killing. The beneficial roles of autophagy in the control of Mtb have been extensively studied by multiple laboratory groups (9, 56–59). Mtb interferes with phagosome maturation by the inhibition of phagosome–lysosome fusion, which results in diminished acidification of the phagosomal compartments, allowing persistence of Mtb in the phagosome. Induction of autophagy by rapamycin or starvation promotes conversion of Mtb phagosomes into autolysosomes, which contain a greater number of antimicrobial compartments (e.g., antimicrobial peptide) than conventional phagolysosomes (9, 56, 60, 61). Autophagy also enhances presentation of mycobacterial antigen in macrophages (58). A recent study identified a key mechanism whereby autophagy targets Mtb in phagocytes such as macrophages (i.e., molecular mechanisms of xenophagy). Extraembryonic spermatogenic homeobox 1 (ESX-1) secretion system (62), a virulence factor of Mtb, promotes the exposure of Mtb DNA to host cytosol by permeabilizing the phagosome membrane (63). The exposed bacterial DNA is recognized by a cytosolic DNA sensor molecule, such as STING, and ubiquitinated (63). The ubiquitinated bacterial DNA binds to LC3 via adaptor protein p62 or nuclear dot protein 52 and is subsequently capsulated in the autophagosome for fusion with lysosome (63).

Autophagy in tuberculosis. Although molecular mechanisms of selective autophagy in Mtb-infected cells have been extensively studied, researchers also expanded studies to explore potential therapeutic approaches for tuberculosis. For example, vitamin D stimulates autophagy activation against Mtb through induction of cathelicidins (64). Interestingly, decreased levels of serum vitamin D are associated with higher risk of active tuberculosis (65). Although recent clinical studies suggest that vitamin D supplementation does not significantly affect time to sputum culture conversion (66, 67), it is also reported that vitamin D reduces time to sputum culture conversion in patients with vitamin D receptor polymorphism (67). The cocktail of isoniazid and pyrazinamide is the first-line standard drug for the treatment of tuberculosis. Intriguingly, isoniazid and pyrazinamide promote

autophagy activation and phagosomal maturation in Mtb-infected host cells, which may be part of a potential mechanisms of these antimycobacterial drugs (59).

Although a number of studies support the significance of autophagy in tuberculosis, a recent study suggests dispensable roles of autophagy in tuberculosis. Myeloid cell-specific ATG5 deficiency increases Mtb replication and reduces the survival in mice infected with Mtb; however, such phenotypes are not observed when other autophagy genes, such as *ATG3*, *ATG7*, *ATG12*, and *ATG16*, are knocked down (68). In addition, ATG5 plays a unique role in protection against Mtb by preventing polymorphonuclear cell-mediated immunopathology (68). These observations suggest that ATG5 may function in nonautophagic processes that contribute to Mtb control in mice. Autophagy in nonimmune cells may be more critically involved in host defense of tuberculosis than that in myeloid cells. Further studies will be necessary to investigate these differential effects of autophagy proteins in tuberculosis models.

Sepsis

Autophagy in sepsis. Although immune responses are essential for host defense against sepsis, excess immune responses and inflammation often trigger tissue/organ injury and subsequent secondary infection (69, 70). The implication of autophagy in sepsis has been suggested by transmission electron microscopy studies on liver samples obtained from patients with sepsis (71). The transmission electron microscopy studies demonstrate the increased number of autophagy vacuoles in the liver of the patients with sepsis compared with patients without sepsis (71). Autophagy activity is assessed by flux of autophagic substrates to the lysosome and degradation of autophagic substrates inside the lysosome (online supplement) (72). Therefore, it is unclear whether this increase of autophagy vacuoles observed in the patients represents increased autophagy activity in patients with sepsis or inhibition of autophagy that led to the inappropriate accumulation of autophagosomes. However, these observations suggest the implication of autophagy in human sepsis.

The significant roles of autophagy have been demonstrated in preclinical models

of sepsis, such as cecal ligation and puncture (CLP)-induced polymicrobial sepsis and an endotoxin-induced septic shock. Autophagy-associated proteins (e.g., LC3B) are up-regulated in major target organs of sepsis, including lung, liver, and kidneys of mice subjected to CLP or endotoxin administration (73, 74). Beneficial roles of autophagy in sepsis models are reported by using various genetically engineered mice or pharmacological autophagy modulators. Genetic depletion of autophagy genes such as *MAP1LC3B*, *BECN1*, and *Vps34* increases inflammation, bacterial burden, organ injury, and mortality in septic mice induced by CLP or LPS (21, 74). In contrast, pharmacological activators of autophagy such as rapamycin or genetic overexpression of LC3 suppresses inflammatory response and apoptotic activity and improves the survival in mice subjected to CLP (75–77). Genetics studies suggest the significance of autophagy in sepsis. For example, polymorphism of immunity-related GTPase M, an important molecule to regulate autophagy induction and eliminate intracellular mycobacteria (78), is associated with the mortality of patients with severe sepsis (79). Carriage of the minor A allele of *ATG16* is also associated with sepsis severity and ventilator-associated pneumonia (80).

Role of selective autophagy in sepsis.

Although regulating inflammation is an important arm to prevent multiorgan dysfunctions in sepsis, proper immune responses are critical to the elimination of microbes during infection. In this respect, autophagy can up-regulate both immune functions (i.e., increasing bacterial killing and suppressing inflammation) mediated by xenophagy and mitophagy. Virulent pathogens killing by xenophagy may contribute to decreasing the number of the pathogens, resulting in immune responses and inflammation during sepsis. In addition to xenophagy, autophagy may also regulate immune responses and inflammation by controlling mitochondrial quality in sepsis. The levels of mtDNA are increased in plasma from patients with sepsis and associated with severity and mortality (81, 82). In contrast, a recent study shows that copy number of mtDNA in monocytes and lymphocytes of patients with sepsis has been shown to decrease and inversely correlate with severity of

illness (e.g., Acute Physiology and Chronic Health Evaluation II score, a commonly used scoring system for a mortality estimation) (83), suggesting that mtDNA can be released from these cells. The release of mtDNA from mitochondria is associated with mtROS-mediated NLRP3 (NLR family, pyrin domain-containing 3) inflammasome activation (21). Thus, these results suggest that mitochondrial integrity may be impaired in human sepsis.

Dysfunctional mitochondria can enhance mitochondrial ROS generation and NLRP3 inflammasome activation, leading to cell death and secretion of proinflammatory cytokines. Autophagy (especially mitophagy) removes those dysfunctional mitochondria to maintain cellular homeostasis. Dysfunctional mitochondria caused by infection or oxidative stress during sepsis can be one of the main causes for immune responses; therefore, eliminating those mitochondria by mitophagy machinery is critical for regulating immune responses and inflammation. In fact, LC3B puncta is increased and colocalized with mitochondria in the distal lung of septic mice induced by *Staphylococcus aureus* infection (84). Functionally, depletion of LC3B and Beclin 1 further increases production of IL-1 β and IL-18 and mortality in septic mice (21). Furthermore, deficiency of Parkin exhibits impaired recovery of cardiac contractility in LPS-treated septic mice (85). The kinase Jnk2 (JNK2)-deficient mice displaying defective mitophagy also cause hyperactivation of inflammasomes and increase mortality in endotoxemic shock (22).

Recent studies suggest deregulation of cellular metabolic pathways, including glycolysis, *de novo* lipid synthesis, and free fatty acid synthesis, is crucial for inflammasome activation, where mitochondria-associated molecules such as hexokinase 1 (HK-1), uncoupling protein-2 (UCP2), and NADPH oxidase 4 (NOX4) are crucially involved (86–88). The immunomodulatory effect of mitophagy may be associated with cellular metabolic changes. Although significant roles of autophagy in immune cells such as macrophages are well documented, it remains unclear whether other types of cells can contribute to the pathogenesis of sepsis. Given the complex nature of pathology in sepsis, further studies using cell-specific target knockout of autophagy genes using lox-system knockout mice or bone marrow transplantation studies (to

generate chimera mice) (68) would be important to determine the roles of autophagy on various cell types/tissues in sepsis.

Idiopathic Pulmonary Fibrosis

Autophagy in idiopathic pulmonary fibrosis. Idiopathic pulmonary fibrosis (IPF) is a progressive fibroproliferative lung disease of unknown cause (89, 90). Unlike COPD, LC3-II expression is decreased in lung tissues from patients with IPF compared with lung from normal subjects (30, 91, 92). In contrast, phosphorylation of S6, an indicator of mTOR activation, is increased in fibroblast foci of IPF lung tissue, suggesting down-regulation of autophagy in IPF (93). In experimental models of IPF, up-regulation of autophagy by rapamycin inhibits transforming growth factor- β -induced fibronectin and α -smooth muscle actin expression in lung fibroblasts (91) and collagen production in lung epithelial cells (94). Similarly, rapamycin also inhibits anti-Toll-like receptor 4 antibody- or bleomycin-induced lung fibrosis in mice (91, 94, 95). In contrast, deficiency of autophagy genes such as *MAP1LC3B*, *ATG5*, and *BECN1* or pharmacological inhibition of autophagy promotes transforming growth factor- β -induced activation of lung fibroblasts (91, 94). Consistently, autophagy inhibition by alveolar epithelial cell-specific knockdown of *TSC1* exacerbates bleomycin-mediated lung injury in mice (93). Thus, autophagy is likely to exert beneficial roles on IPF models.

Selective autophagy in IPF. A recent study has revealed the accumulation of dysmorphic and dysfunctional mitochondria in lung epithelial cells, especially alveolar type II cells (AECIIs), from the lungs of patients with IPF (96). High mitochondrial content was observed, especially in AECIIs lining the areas of honeycombs and dense fibrosis, whereas no differences in mitochondrial content were observed in airway epithelial cells between IPF and donor control lungs (96). Microarray analyses showed the decrease of *PINK1* expression in lung samples from patients with IPF (96), and this down-regulation of *PINK1* was observed in AECIIs of patients with IPF but not in the fibroblasts (96). These observations suggest deregulated mitochondria homeostasis and also implication of selective autophagy targeting mitochondria in IPF.

Functionally, genetic knockdown of

PINK1 displays increased mitochondria depolarization and expression of profibrotic factors in mice (96, 97). The mechanism of antifibrotic effect by *PINK1* may include prevention of cell death by preserving mitochondrial function of pulmonary epithelial cells (96, 97). In addition, Parkinson protein 2 E3 ubiquitin protein ligase (*PARK2*), another key mitophagy-associated molecule, is also implicated in myofibroblast differentiation and proliferation (98), although function of *PARK2* on lung fibrosis needs to be further investigated (98, 99). It remains unclear whether the observed accumulation of dysfunctional mitochondria and defective mitophagy in the lung of patients with IPF is a consequence of IPF or cause of IPF. Unlike CS-associated COPD, various factors are associated with onset of IPF. Further studies are necessary to investigate the upstream mechanism by which mitophagy is regulated in IPF.

Autophagy as a Potential Therapeutic Target

Drugs modulating autophagy, such as rapamycin or chloroquine, have been clinically used for many years (100, 101). These autophagy modulators are also now tested in clinical trials targeting pulmonary diseases, including lung cancer (102). The details of ongoing clinical trials targeting modulating autophagy are listed at <https://www.clinicaltrials.gov/ct2/results?term=autophagy>. Currently, more than 30 different classes of drugs to modulate autophagy activity have been synthesized and identified (102–104). Of note, new-class autophagy modulators such as temsirolimus/CCI-779 or everolimus (mTORC1 inhibitors) display improved specificity and effectiveness and have been recently approved for the treatment of cancer (105). Recent studies suggest use of selective autophagy modulators such as Mdivi-1 can regulate pathological conditions in experimental pulmonary diseases (14, 106). The development of modulators targeting each selective autophagy pathway may be also beneficial, as those modulators can avoid unnecessary manipulation of other autophagy pathways, therefore having fewer off-target effects.

However, there are several important points to be further investigated. For example, effective and less-invasive methods

to monitor autophagy activity for patients (e.g., biomarkers) are not currently available. In addition, because of its fundamental cellular process and functions, it is plausible that modulating autophagy causes other untoward effects that may be clinically deleterious. Although autophagy has been shown to be beneficial for infectious diseases, up-regulation of autophagy may be harmful in certain disease conditions, such as COPD or cancer. It is also important to consider that each cell type may have distinct autophagy regulation and display unexpected cellular responses to autophagy modulators. Autophagy is up-regulated in lung epithelial cells in response to CS (13, 30), whereas autophagy activity is suppressed in CS-exposed alveolar macrophages (38). If patients with COPD are to receive intervention targeting down-regulation of autophagy, there is a possibility that the interventions may cause defective bacterial killing in alveolar macrophages and lead to respiratory infections and acute exacerbation of COPD.

Considering the beneficial aspects of autophagy, such as in host defense and even antiaging effects (online supplement), the modulation of autophagy (including selective autophagy) may serve as a potential intervention for various human diseases. Further understanding of cell-specific and/or selective autophagy regulation and better technologies of autophagy manipulation with minimal off-target effect may promote the development of optimized autophagy modulators.

Conclusions

The pathophysiologic role of autophagy has been extensively studied as a potential new therapeutic target. As described in this review, autophagy is involved in the pathogenesis of various preclinical models of pulmonary diseases and is likely to be regulated in human pulmonary diseases. Although autophagy has been initially believed to serve to act as a cytoprotective process in most host responses, recent

studies suggest the diverse functions of autophagy in lung diseases, including potentially deleterious effects in specific pathophysiological states, such as COPD, cancer, and avian influenza virus-mediated pneumonia (Figure 6 and Table E2). In addition, the recent recognition of molecular regulation and function of specialized autophagy is of significance. During the last 5 years, the implication of selective autophagy in pulmonary diseases has been extensively reported. Of note, multiple selective autophagy pathways can uniquely regulate pathogenesis of each disease. Implication of selective autophagy in human diseases may be more complex but also substantially important. Studies of the regulation and roles of each selective autophagy may reveal new pathological mechanisms of human diseases, which may lead to potential therapeutic targets. ■

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