Autophagy: a potential therapeutic target in lung diseases

Kiichi Nakahira and Augustine M. K. Choi

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts

Submitted 18 March 2013; accepted in final form 17 May 2013

Nakahira K, Choi AM. Autophagy: a potential therapeutic target in lung diseases. Am J Physiol Lung Cell Mol Physiol 305: L93–L107, 2013. First published May 24, 2013; doi:10.1152/ajplung.00072.2013.—Macroautophagy (hereafter referred to as autophagy) is an evolutionally conserved intracellular process to maintain cellular homeostasis by facilitating the turnover of protein aggregates, cellular debris, and damaged organelles. During autophagy, cytosolic constituents are engulfed into double-membrane-bound vesicles called “autophagosomes,” which are subsequently delivered to the lysosome for degradation. Accumulated evidence suggests that autophagy is critically involved not only in the basal physiological states but also in the pathogenesis of various human diseases. Interestingly, a diverse variety of clinically approved drugs modulate autophagy to varying extents, although they are not currently utilized for the therapeutic purpose of manipulating autophagy. In this review, we highlight the functional roles of autophagy in lung diseases with focus on the recent progress of the potential therapeutic use of autophagy-modifying drugs in clinical medicine. The purpose of this review is to discuss the merits, and the pitfalls, of modulating autophagy as a therapeutic strategy in lung diseases.

MOLECULAR MECHANISM OF AUTOPHAGY

Autophagy encloses cytosolic materials by isolation membranes (phagophores) to form double membrane-bound vesicles called “autophagosomes” (Fig. 1). The isolation membranes are acquired from multiple sources including endoplasmic reticulum, Golgi apparatus, mitochondrial outer membrane, and plasma membrane (35, 76, 93, 116). Subsequently, the autophagosome containing the cytosolic components and organelles fuses with the lysosome to become autolysosome, with subsequent degradation of cytosolic components (Fig. 1). More than 30 autophagy-related gene (Atg) proteins have been identified to be involved in autophagosome formation (73, 94). The activation of autophagy is mainly regulated by two signaling pathways including mammalian target of rapamycin (mTOR)-dependent and mTOR-independent pathways (94). In normal physiological condition, autophagy activity is regulated at basal level by activation of mTOR, a serine/threonine kinase that has diverse cellular functions. When cells encounter nutritional starvation, mTOR is inactivated and promotes the autophagosome formation (Fig. 2, left). In mTOR-independent pathways, Ca2+—calpain–Gsa and cyclic AMP (cAMP)–phospholipase Cε (PLCε)–inositol-(1,4,5)-trisphosphate (IP3) pathways are involved in autophagy activation (Fig. 2, right).

FUNCTION OF AUTOPHAGY

 Whereas autophagy nonselectively engulfs and degrades intracellular proteins for recycling under nutritional starvation, autophagy can selectively target and remove specific subcellular components (selective autophagy) (18). This selective degradation pathway includes eliminating invading pathogens (xenophagy) (60), dysfunctional cellular organelles such as mitochondria (mitophagy) (126), and polyubiquitinated protein aggregates (aggrephagy) (56). Autophagy is also involved in lipid metabolism (lipophagy) (108). Selective autophagy
plays important roles in maintaining cellular homeostasis in basal physiological states and in response to various cellular stresses.

Cytoprotective roles of autophagy under stress conditions such as starvation have been well documented (73). However, when cells receive lethal signals, the cellular stresses cause autophagy and also cell death including apoptosis. Although interactions of autophagy- and apoptosis-related molecules such as Beclin 1 and B cell lymphoma 2 (BCL-2), or LC3B and Fas have been reported in various models (16, 87), it is still unclear whether autophagy induced by lethal signals promotes cell death or is an independent process from cell death (94). The functional role of autophagy on cell death is likely to be dependent on stress models.

Against invading microbes, autophagy actively participates in innate immune responses (54, 60). For example, autophagy exerts its host defense role by degrading various pathogens by lysosomal system (xenophagy) (60). The target pathogens include bacteria, such as group A Streptococcus pyogenes (60), Mycobacterium tuberculosis (MtB) (23), Salmonella enterica (54), and Pseudomonas aeruginosa (127); viruses such as herpes simplex virus 1 (54, 60); and parasites such as Toxoplasma gondii (54). In contrast, recent studies also suggest that autophagy machinery contributes to the replication and survival of microbes in the host cells. The list of pathogens exploiting autophagy machinery includes clinically important microbes, e.g., bacterial agents such as Brucella abortus (99) and Coxiella burnetii (99) and viruses such as HIV (99), hepatitis B virus (HBV) (54, 99), and avian influenza A H5N1 (H5N1) (109). Of note, autophagy exerts both killing and prosurvival properties for HIV (60, 99). Autophagy has diverse effects on both innate and adaptive immune systems (54, 60). Atg5 is involved with the production of type I interferons in response to single-stranded RNA viruses (54, 60). Autophagy proteins are also involved in the regulation of inflammasome, cytosolic multiprotein complexes responsible for activation of caspase-1 and its downstream signaling including secretion of IL-1β and IL-18 (20, 79). Beclin 1 and LC3B negatively regulate inflammasome activation by preserving mitochondrial homeostasis (79). The roles of autophagy in adaptive immunity include antigen presentation and antibody production. Autophagy is required for presenting antigen on both major histocompatibility complex class I and II molecules and activating CD8+ and CD4+ T lymphocytes, respectively (60). Autophagy is also required for B cell differentiation (19) and for sustainable antibody production in plasma cells (88). Thus
Autophagy plays crucial diverse roles in immune systems including pathogen degradation and immune signaling, suggesting that the involvement of autophagy in infectious and inflammatory diseases.

Autophagy is critically involved in various metabolic pathways for maintaining cellular homeostasis (91). For example, autophagy is an important energy generator in the liver. Mice with specific deletion of \textit{atg7} gene in liver display decreased level of blood glucose and amino acids after starvation (25). Autophagy is also involved in lipid metabolism by breaking down lipid droplets to generate energy (lipophagy) (108). Metabolome analysis also suggests autophagy is required for maintenance of tricarboxylic acid cycle-related metabolites (33), suggesting that autophagy is involved in the quality control of mitochondria. Damaged mitochondria are selectively targeted and removed by autophagy to maintain normal mitochondrial function (mitophagy) (126). Although the concise molecular mechanism of mitophagy is still unclear, the importance of preserving mitochondrial homeostasis by autophagy is now further linked to the pathogenesis of human

---

**Fig. 2.** Overview of the regulation of autophagy pathways and targets for modulating autophagy by drugs/compounds. Two major signaling pathways to regulate autophagy are depicted in this figure: mammalian target of rapamycin (mTOR) signaling pathways and mTOR-independent signaling pathways. Autophagy is modulated by mTOR in response to certain nutritional stimulations. Insulin or growth factors activate class I PI3K pathway, leading to activation of mTOR and inhibition of autophagy. Glucose starvation activates AMPK and inhibits mTOR, followed by activation of ULK1 complex and activating autophagy. mTOR can be pharmacologically inhibited by activating AMPK (e.g., metformin) or inhibiting class I PI3K (e.g., EGFR antagonist). Recent new drugs such as PI-103 can activate autophagy by inhibiting both class I PI3K and mTOR. In the mTOR-independent pathway, increase of intracellular cAMP level and Ca\textsuperscript{2+} inhibits autophagy. Intracellular level of cAMP is upregulated by adenylyl cyclase (AC) and the calpain-Gs \textit{pathway}, which leads inhibition of autophagy. Activation of L-type Ca\textsuperscript{2+} channel triggers \textit{pathway}, which is activated by intracellular Ca\textsuperscript{2+} level. The inhibitory effect of cAMP on autophagy is mediated by increase of synthesis of inositol-(1,4,5)-trisphosphate (IP3) and inositol. Increased level of IP3 activates IP3 receptor in endoplasmic reticulum (ER) and release Ca\textsuperscript{2+} into cytosol, leading to inhibition of autophagy. Activation of L-type Ca\textsuperscript{2+} channel triggers \textit{pathway}, which is activated by intracellular Ca\textsuperscript{2+} level. In addition to elevating cAMP by the calpain-Gs \textit{pathway}, calpain inhibits autophagy by cleavage of Atg5. However, the concise mechanism by which cytosolic AMP, Ca\textsuperscript{2+}, inositol, and IP3 regulate autophagy is still unclear. In this cyclcal mTOR-independent pathway, autophagy can be modulated by mainly targeting intracellular levels of cAMP or Ca\textsuperscript{2+}. L-type Ca\textsuperscript{2+} receptor blockers such as verapamil inhibit Ca\textsuperscript{2+} influx, which leads inhibition of calpain activity and cAMP synthesis. Activation of autophagy by drugs such as lithium or carbamazepine is mediated by at least reduction of Ca\textsuperscript{2+} release by IP3 receptor (IP3R). EGFR, epidermal growth factor receptor; IGF, insulin-like growth; TSC, tuberous sclerosis 2; AMPK, AMP-activated protein kinase; V-ATPase, vacuolar ATPase; I1R, imidazoline-1 receptor; PLC, phospholipase C epsilon; 2'5-ddA, 2'5'-dideoxyadenosine; PIP2, phosphatidylinositol-4,5-bisphosphate; cAMP, cyclic AMP; Ins, inositol; IP2, inositol-(1,4,5)-trisphosphate; IMPase, inositol monophosphatase.
diseases such as Parkinson disease (68, 80, 81, 118). Thus the autophagy process is critically involved in not only physiological but also pathological metabolic responses.

MEASUREMENT OF AUTOPHAGY

As the research of autophagy continues to evolve, methods for monitoring autophagy have been evaluated and discussed in detail. Klionsky et al. (51) have recently reported the guideline for monitoring autophagy in higher eukaryotes and described useful methods and how to interpret the data. A key tenet to emphasize is that investigators need to recognize whether they are evaluating the steady-state levels of autophagosomes or dynamic state of autophagy generated by their models (51, 101). If they are seeking to assess the change of autophagy flux or activity (e.g., the rate of delivery of autophagosomes substrate to lysosomes) in certain points, it is likely that monitoring steady state of autophagosome (e.g., measuring LC3-II expression without examining turnover by Western blot, counting autophagosomes by using electron microscopy, or counting puncta formation of LC3 by immunofluorescence microscopy) is not sufficient. Table 1 demonstrates the various methods to monitor autophagy both in vitro and in vivo. Generally, it is recommended to use multiple different assays (ideally for both steady and dynamic states), rather than relying on the results from a single method (51, 101).

Table 1. Methods for monitoring autophagy in vitro and in vivo

<table>
<thead>
<tr>
<th>Methods</th>
<th>Key Points</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monitoring static state autophagy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quantifying autophagosomes</td>
<td>Electron microscopy</td>
<td>• Positive correlation of elevated number of autophagosomes and increased autophagy flux has not been proved reliably in all models. 110</td>
</tr>
<tr>
<td>LC3-II level (ratio of LC3-II/LC3-I)</td>
<td>Western blot</td>
<td>• Increased level of LC3-II (ratio of LC3-II/LC3-I) does not always correlate with autophagy flux. 75</td>
</tr>
<tr>
<td>Puncta formation of LC3</td>
<td>Immunofluorescence microscopy</td>
<td>• This method can be applied to in vivo using GFP-LC3 transgenic mice. 72, 74 • Increased puncta formation of LC3 does not reliably correlate with autophagic flux.</td>
</tr>
<tr>
<td>Monitoring dynamic state autophagy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turnover of LC3-II</td>
<td>Western blot</td>
<td>• LC3-II on the autophagosome membrane is normally continuously degraded during autophagy process. LC3-II can be accumulated by adding lysosomal proteolysis inhibitors such as leupeptin, chloroquine or bafilomycin. Cells or animals can be treated with lysosomal protease inhibitors. Autophagic flux can be expressed as the difference in LC3B-II signal on Western blot obtained in the presence or absence of lysosome protein inhibitors. 38, 41</td>
</tr>
<tr>
<td>Turnover of p62/SQSTM1</td>
<td>Western blot</td>
<td>• p62/SQSTM1 is a cytosolic protein that has an LC3 binding domain (REF). It binds to ubiquitinated protein and carry them to autophagosome. Subsequently, both p62/SQSTM1 and the cargo proteins are degraded by autophagy. • Similar with LC3-II, p62/SQSTM1 turnover can be measured by adding lysosome protease inhibitors in vitro or in vivo. 10, 38</td>
</tr>
<tr>
<td>Cytosolic protein sequestration assays</td>
<td>Western blot</td>
<td>• Cells are incubated (or animals are intraperitoneally administered) with leupeptin to inhibit lysosomal proteolysis. Autophagosomes are purified via mechanical disruption (34) followed by density centrifugation. Autophagy flux is expressed by the amount of cytosolic protein, such as LDH recovered in the autophagosome fraction (Western blot). 38, 52, 106</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase.

AUTOPHAGY PATHWAY: POTENTIAL THERAPEUTIC TARGET FOR LUNG DISEASES

Autophagy in Models of Human Lung Diseases

The functional roles of autophagy on various lung diseases have been studied both in vitro and in vivo. Tables 2 and 3 represent the functions of autophagy or autophagic proteins in lung diseases on the basis of studies using genetic or biochemical perturbation of autophagy. Although autophagy has been initially thought as a cytoprotective response in pathophysiological states, accumulating data reveal diverse functions of autophagy in lung diseases (Tables 2 and 3). For example, chronic obstructive pulmonary disease (COPD) is one lung disease that has been shown to be associated with autophagy (15). Increased number of autophagosomes is observed by electron microscopy analysis, and expression of autophagic protein LC3B-II is increased in lung tissues from patients with COPD (15). Several molecules involved in autophagy-mediated COPD include FAS (16), Toll-like receptor 4 (5), and cystic fibrosis transmembrane conductance regulator (CFTR) (11). In addition, alveolar macrophages isolated from smokers show increased of LC3 expression with defect of autophagy flux (77). In vivo, genetic deletion of LC3B displays resistance to cigarette smoke-induced airway space enlargement compared with the control mice (16). Similar adverse effects of
### Table 2. The functional role of autophagic proteins in experimental models of lung diseases

<table>
<thead>
<tr>
<th>Lung Diseases</th>
<th>Function of Autophagic Proteins</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>• LC3B and Beclin 1 deficiency inhibits CSE-induced cell death in pulmonary epithelial cells. LC3B-deficient mice display inhibition of airspace enlargement and apoptosis in lungs after cigarette smoke exposure.</td>
<td>15, 16, 47</td>
</tr>
<tr>
<td></td>
<td>• LC3 deficiency promotes CSE-induced IL-8 secretion in HBEC.</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>• Autophagy inhibition by baflomycin A enhances CSE-induced IL-8 secretion in HBEC.</td>
<td></td>
</tr>
<tr>
<td>IPF</td>
<td>• Autophagy activation by rapamycin inhibits IL-17A-induced collagen production in lung epithelial cells.</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>• Autophagy inhibition by 3-MA suppresses degradation of collagen in epithelial cells. 3-MA reverses the therapeutic effect of IL-17 antagonism on bleomycin-induced lung fibrosis and mortality of mice.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• LC3B and Beclin 1 deficiency promotes TGF-β-induced activation of lung fibroblast in vitro. Autophagy activation by rapamycin inhibits TGF-β-induced fibronectin and α-SMA expression in lung fibroblast. Rapamycin inhibits bleomycin-induced lung fibrosis in mice.</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>• LC3B knockdown increases serum level of IL-1β and IL-18 production in CLP-induced polymicrobial sepsis and sensitizes mice to endotoxic shock.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• LC3B knockdown increases serum level of IL-1β and IL-18 production in CLP-induced polymicrobial sepsis and sensitizes mice to endotoxic shock. Autophagy activation by rapamycin restores CLP-induced myocardial dysfunction in mice.</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>• LC3 overexpression ameliorates acute lung injury and survival in CLP-induced polymicrobial model of sepsis.</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>• Knockdown of VPS34 increases cell death and liver injury in CLP-induced polymicrobial models of sepsis.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>• Autophagy inhibition suppresses releases of neutrophil extracellular trap (NET) induced by E. coli in human neutrophils.</td>
<td>45</td>
</tr>
<tr>
<td>PH (PAH)</td>
<td>• LC3B deficiency promotes hypoxia-induced pulmonary hypertension in mice. LC3B deficiency promotes cell proliferation in pulmonary artery endothelial cells and vascular smooth muscle cells. Autophagy inhibition by 3-MA and chloroquine increases angiogenesis in PAEC from fetal lambs with persistent pulmonary hypertension (PPHN-PAEC). Beclin-1 knockdown promotes angiogenesis in PPHN-PAEC.</td>
<td>59, 114</td>
</tr>
<tr>
<td>CF</td>
<td>• Beclin 1 deficiency promotes aggresome accumulation in CF epithelia. Beclin 1 deficiency promotes macrophage infiltration and MPO activity in nasal mucosa of CF. Autophagy inhibition by 3-MA abrogates CF phenotype in nasal mucosa.</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>• LC3 deficiency promotes growth of B. cepacia and IL-1β secretion in macrophages. Autophagy activation by rapamycin inhibits growth of B. cepacia and IL-1β secretion in macrophages. Autophagy activation reduces bacterial burden of lung and inflammation in lung of CF mice after B. cepacia infection.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>• Overexpression of SQSTM1/p62 inhibits intracellular survival of B. cepacia in macrophages. Deficiency of SQSTM1/p62 promotes intracellular survival of B. cepacia in macrophages.</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>• Autophagy inhibition by chloroquine enhances cytotoxicity of gefitinib and erlotinib in lung cancer cells. Atg5 and Atg7 deficiency enhances cytotoxicity of gefitinib and erlotinib in lung cancer cells.</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>• Atg7 deficiency reduces cancer cell proliferation in NSCLC cell lines. Atg7 deficiency sensitizes the cancer cells to cisplatin-induced apoptosis in NSCLC cell lines.</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>• Atg3 deficiency reverses erlotinib resistance in erlotinib-resistant lung cancer cells.</td>
<td>57</td>
</tr>
<tr>
<td>LAM</td>
<td>• TSC2 knockdown increases autophagy-dependent cell survival in mouse embryonic fibroblasts. Atg5 deficiency inhibits TSC2-null xenograft tumor cell survival. Autophagy inhibition by chloroquine inhibits xenograft tumor size in mice of TSC2-null xenograft tumor.</td>
<td>84</td>
</tr>
<tr>
<td>HALI</td>
<td>• LC3B knockdown promotes hyperoxia-induced cell death in lung epithelial cells.</td>
<td>112</td>
</tr>
<tr>
<td>Sepsis</td>
<td>• Depletion of LC3B and Beclin 1 leads mitochondrial dysfunction and enhances NLRP3 inflammasome-mediated IL-1β and IL-18 secretion in macrophages. LC3B knockdown increases serum level of IL-1β and IL-18 production in CLP-induced polymicrobial sepsis and sensitizes mice to endotoxic shock.</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>• Autophagy activation by rapamycin restores CLP-induced myocardial dysfunction in mice.</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>• LC3 overexpression ameliorates acute lung injury and survival in CLP-induced polymicrobial model of sepsis.</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>• Knockdown of VPS34 increases cell death and liver injury in CLP-induced polymicrobial models of sepsis.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>• Autophagy inhibition suppresses releases of neutrophil extracellular trap (NET) induced by E. coli in human neutrophils.</td>
<td>45</td>
</tr>
<tr>
<td>Nanoparticle-induced ALI</td>
<td>• Knockdown of Atg6 improves nanoparticle-induced cell death in lung epithelial cells. Autophagy inhibition by 3-MA ameliorates nanoparticle-induced ALI in mice.</td>
<td>61, 62</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease; CSE, cigarette smoke extract; HBEC, human bronchial epithelial cells; IPF, idiopathic pulmonary fibrosis; TGF-β, transforming growth factor-β; α-SMA, α-smooth muscle actin; 3-MA, 3-methyladenine; TLR4, Toll-like receptor 4; ER, endoplasmic reticulum; PAE, pulmonary artery endothelial cells; CF, cystic fibrosis; MPO, myeloperoxidase; B. cepacia, Burkholderia cenocepacia; NSCLC, nonsmall cell lung cancer; LAMP2, lysosome-associated membrane protein 2; LAM, Lymphangioleiomyomatosis; TSC, tuberous sclerosis; HALI, hyperoxia-induced acute lung injury; NLRP3, NOD-like receptor family, pyrin domain containing 3; CLP, cecal ligation and puncture; E. coli, Escherichia coli; ALI, acute lung injury.
**Autophagy in Clinical Trials**

Although drugs modulating autophagy such as rapamycin or chloroquine have been clinically used for years without the intent to modulate autophagy activity, these drugs are now studied as autophagy modulators in clinical trials for lung diseases (17, 99, 120, 125). For example, chloroquine, which inhibits autophagy-mediated cell survival in tumor cells, is used as an intervention for patients with small cell lung cancer in a clinical trial (phase 1). In addition, the interventions using hydrochloroquine and other anticancer drugs such as erlotinib are used for non-small cell lung cancer in two clinical trials (phase 1/2 and 2) (125). Although autophagy has been initially shown to be associated with anti-tumorigenesis in rodent animal studies (90, 111), the current strategy for cancer therapy is more likely to be based on inhibition of autophagy (17, 120, 125).

This paradox may be due to the differential roles of autophagy in different stages of tumorigenesis (99, 120). Although initiation of tumorigenesis in normal cells is associated with defect of autophagy, autophagy subsequently may exert prosurvival effect in tumor cells (99, 120). Lymphangioleiomyomatosis (LAM), a progressive lung disease caused by mutation in the tuberous sclerosis genes (tsc), is associated with inappropriate activation of mTOR signaling, which regulates cellular growth and lymphangiogenesis (39). The pathogenesis of LAM is in part similar with those of tumorigenesis including inappropriate cell growth and survival (39). Chloroquine is also used with rapamycin for patients with LAM (phase 1). This clinical trial is based on the rationale that rapamycin blocks mTOR of downstream kinases and restores homeostasis in cells with defective tsc function (39, 69, 84). The use of chloroquine aims to inhibit autophagy-mediated survival of LAM cells induced by rapamycin (39, 84). A recent clinical study concludes chloroquine does not prevent infection with influenza including H1N1 strain (86). Interestingly, autophagy is involved in infection with H5N1, rather than H1N1 (109). Therefore, it is possible that chloroquine has a differential effect on infection with H5N1 vs. H1N1, with genetic and...
biochemical inhibition of autophagy rescue in mice infected with H5N1 (109). Since chloroquine also has autophagy-independent pharmacological effects such as anti-inflammatory property, the effect of chloroquine on diseases including tumor may not be entirely explained by modulating autophagy (120). Although further studies are needed to evaluate the off-target effects of chloroquine on diseases, these reports suggest that autophagy modulation by clinically used drugs may be more accessible to study in clinical trials because of their favorable safety profiles. The details of ongoing clinical trial targeting modulating autophagy are listed (http://www.clinicaltrials.gov/ct2/results?term=autophagy&Search=Search).

**Autophagy Modulators**

Given the important association between autophagy and human lung diseases, it is worthwhile to review whether modulating autophagy can serve as potent therapeutic targets for various lung diseases. Table 4 demonstrates a list of autophagy modulating drugs that are clinically used or being studied in clinical trials. Table 5 shows conventional and newly developed compounds or drugs such as L-

- **LAM**
  - Promoting survival and proliferation of LAM cells
  - Cystic fibrosis
  - Clearance of protein aggregate and regulating oxidative stress

- **Lung cancer**
  - Promoting survival and proliferation of cancer cells
  - Tumor suppression

- **Aggrephagy**
  - Promoting or inhibiting replication of microbes in host cells

- **Xenophagy**
  - Promoting or inhibiting replication of microbes in host cells
  - Regulating inflammatory response (i.e., inflammasome)

- **Mitophagy**
  - Mitochondria (mitophagy)

- **Lipophagy**
  - Promoting or inhibiting replication of microbes in host cells

- **IPF**
  - Regulating fibroblast activation and proliferation

- **COPD**
  - Promoting apoptosis

- **PAH**
  - Regulating vascular cell proliferation

**Fig. 3. Diverse effects of autophagy in lung diseases.** The functional modes of autophagy at cellular levels include eliminating intracellular microbes (xenophagy), dysfunctional mitochondria (mitophagy), and protein aggregate (aggrephagy). Autophagy also regulates lipid metabolism (lipophagy). Autophagy is involved in pathogenesis of various lung diseases. The roles of autophagy in each disease are shown. In lung cancer, autophagy may prevent tumorigenesis, whereas autophagy can promote survival and proliferation of tumor cells. In infectious diseases, the roles of autophagy are dependent on microbes. Autophagy may promote bacterial killing and inhibit intracellular survival of microbes such as *Mycobacterium tuberculosis*. On the other hand, autophagy may promote replication and survival of microbes such as H5N1.

**mTOR signaling pathways.** The mTOR is one of the main targets for modulating autophagy by drugs and forms two distinct complexes to regulate autophagy: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (94) (Fig. 2, left). Rapamycin and its analogs inhibit mTORC1, a main complex responsible for autophagy regulation, and promote autophagy induction (92, 95, 121). A recently identified compound Torin1 directly inhibits both mTORC1 and mTORC2 and induces greater autophagy than does rapamycin (115). Of note, another new class of drugs has dual targets in autophagy pathways. PI-103 hydrochloride inhibits both mTOR and class I phosphoinositide 3-kinase (class I PI3K), suggesting an effective autophagy inducer (22).

**mTOR-independent pathways.** Figure 2, right, shows mTOR-independent pathways.

**PHOSPHOINOSITOL SIGNALING PATHWAY.** Intracellular inositol and IP3 levels negatively regulate autophagy (94). Drugs such as lithium (103) or carbamazepine (103, 121) increase autophagy activity by decreasing intracellular level of inositol.

**Ca2+-CAMP-PLCE-IP3 PATHWAY.** Intracellular Ca2+ and cAMP negatively regulate autophagy by increasing intracellular inositol level (94). In addition, calpain activated by elevated level of intracellular Ca2+ inhibits autophagy by cleavage of Atg5 (94, 99). Antihypertensive drugs including Ca2+ receptor blockers (9, 121, 129) or imidazolin receptor agonists (98, 121) induce autophagy by decreasing cAMP.

**Degradation steps of autophagy.** Inhibiting formation of autolysosome and lysosomal protease are also important targets to inhibit autophagy (Fig. 1). Chloroquine is a lysosomotropic and inhibits the fusion of autophagosome and lysosome (4, 12). Cystatin B is a potent inhibitor of cystatin protease (cathepsin B) in lysosome (99).

**Other pathways.** BCL-2 homology 3 (BH3) mimics inhibit the interaction of BCL-2 and BCL-x with Beclin 1, an inhibitory complex for autophagy (66) (Fig. 1). Resveratrol induces autophagy by activating sirtuin 1 and inhibits P70 S6 kinase (8, 43, 82). There are several compounds or drugs such as L-
### Table 4. Drugs that modulate autophagy activity

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Clinical Application or Pharmacological Class</th>
<th>Mechanism of Action in Autophagy Pathway</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autophagy inducers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapamycin and analogs</td>
<td>Immunosuppressant for preventing rejection in organ transplant and coronary stent coating for anti-proliferation</td>
<td>Inhibit mTORC1</td>
<td>92, 95, 121</td>
</tr>
<tr>
<td>Amidarone</td>
<td>Class III antiarrhythmic</td>
<td>Inhibits mTORC1 or upstream in mTOR pathway</td>
<td>9</td>
</tr>
<tr>
<td>Nicosamide</td>
<td>Antiparasitic</td>
<td>Inhibits mTORC1 or upstream in mTOR pathway</td>
<td>9</td>
</tr>
<tr>
<td>EGFR antagonists (erlotinib hydrochlorine)</td>
<td>Anticancer</td>
<td>Inhibit PI3K-Akt-mTOR pathway</td>
<td>30, 36</td>
</tr>
<tr>
<td>Resveratrol (Stilbenoids)</td>
<td>Supplementary diet. Secondary products of heartwood formation in trees that act as phytalexins</td>
<td>Activates sirtuin 1 (histone deacetylase) and inhibits S6 kinase</td>
<td>8, 43, 82</td>
</tr>
<tr>
<td>Suberoylanilide hydroxamic acid</td>
<td>Anticutaneous T cell lymphoma</td>
<td>Inhibits mTOR</td>
<td>13</td>
</tr>
<tr>
<td>Dexamethasone (glucocorticoid)</td>
<td>Anti-inflammatory or immunosuppressant</td>
<td>Upregulates PML and Akt dephosphorylation</td>
<td>31, 55</td>
</tr>
<tr>
<td>Metformin</td>
<td>Antidiabetic</td>
<td>Upregulates AMPK (and ULK1 phosphorylation)</td>
<td>48, 70</td>
</tr>
<tr>
<td>Verapamil, nicardipine, nimodipine (L-type Ca\textsuperscript{2+} channel blockers)</td>
<td>Vasodilator</td>
<td>Reduce intracellular Ca\textsuperscript{2+} levels</td>
<td>9, 121, 129</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>Anticonvulsant</td>
<td>Inhibits histone deacetylase and reduces intracellular Ca\textsuperscript{2+} levels</td>
<td>103, 121</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Antihypertensive, treatment for ADHD and anxiety/panic disorder</td>
<td>Reduces cAMP levels (Imidazoline-1 receptor agonist)</td>
<td>121</td>
</tr>
<tr>
<td>Rilmenidine</td>
<td>Antihypertensive</td>
<td>Reduces CAMP levels (imidazoline-1 receptor agonist)</td>
<td>98, 121</td>
</tr>
<tr>
<td>Lithium, L-690 330</td>
<td>Mood-stabilizing drug</td>
<td>Inhibit IMPase and reduce inositol and IP3 levels</td>
<td>103</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Antiepileptics</td>
<td>Reduces inositol and IP3 levels</td>
<td>103, 121</td>
</tr>
<tr>
<td>Tamoxifen (estrogen receptor antagonist)</td>
<td>Anti-breast cancer and anti-bipolar disorder</td>
<td>Accumulation of sterol, Increases Beclin 1</td>
<td>21, 99</td>
</tr>
<tr>
<td>Statin (HMG-CoA reductase inhibitor)</td>
<td>Lower cholesterol</td>
<td>Depletes geranylgeranyl diphosphate</td>
<td>83</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Supplementary diet to reduce the risk of fractures and falls</td>
<td>Increases Beclin 1 expression by upregulating calcitcin</td>
<td>128</td>
</tr>
<tr>
<td>BH3 mimetics (ABT-737)</td>
<td>Undergoing clinical trial for ovarian cancer</td>
<td>Disrupts interaction between the BH3 domain of Beclin 1 and the ant apoptotic proteins BCL-2</td>
<td>66</td>
</tr>
<tr>
<td>Carbon monoxide (at 250 ppm)</td>
<td>Vasodilator, anti-inflammation and antiproliferation Undergoing clinical trials for idiopathic pulmonary fibrosis and sepsis</td>
<td>Increases mitochondrial reactive oxygen species</td>
<td>58</td>
</tr>
<tr>
<td>Minoxidil (potassium channel opener)</td>
<td>Macrolide antibiotic</td>
<td>Inhibits fusion of autophagosome and lysosome</td>
<td>96</td>
</tr>
<tr>
<td>Salbutamol (β\textsubscript{2} adrenergic receptor agonist)</td>
<td>Treatment for asthma by smooth muscle relaxant</td>
<td>Inhibits fusion of autophagosome and lysosome</td>
<td>4, 12</td>
</tr>
<tr>
<td>Isoniazid, pyrazinamide</td>
<td>Antibiotics for tuberculosis</td>
<td>AMPK and intracellular Ca\textsuperscript{2+} dependent</td>
<td>49</td>
</tr>
<tr>
<td><strong>Autophagy inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Macrolide antibiotic</td>
<td>Inhibits fusion of autophagosome and lysosome</td>
<td>4, 12</td>
</tr>
<tr>
<td>Chloroquine/hydroxychloroquine</td>
<td>Treatment for RA, SLE, and malaria Undergoing clinical trials for various cancers</td>
<td>Inhibits microtubule formation</td>
<td>89</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>Anticancer</td>
<td>Activates Akt and STAT6</td>
<td>37</td>
</tr>
<tr>
<td>IL-4</td>
<td>Undergoing clinical trial for tuberculosis by blocking IL-4</td>
<td>Activates Akt and STAT6</td>
<td>37</td>
</tr>
</tbody>
</table>

mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; PI3K, phosphoinositide 3-kinase; NF-κB, nuclear factor-κB; AMPK, AMP-activated protein kinase: ULK1, UNC51-like kinase; cAMP, cyclic AMP; IP3, inositol-(1,4,5)-trisphosphate; IMPase, inositol monophosphatase; BH3, BCL-2 homology 3; B cell lymphoma 2; EGFR, epidermal growth factor receptor; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; STAT6, signal transduction and transcription 6; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; PML, promyelocytic leukemia protein; ADHD, attention deficit hyperactivity disorder; IL-4, interleukin 4; IL-13, interleukin 13. For details of clinical trials, please see the website http://www.clinicaltrials.gov/.
In addition, some drugs may inhibit Ca\(^{2+}\) activate mTOR and lead to inhibition of autophagy (27). Although lithium is possible that some drugs can stimulate both proautophagy and antiautophagy pathways. For example, although lithium can also inhibit EGFR receptor (inactivating autophagy by mTOR-independent pathways) and activate mTOR (activating autophagy by mTOR-independent pathways) and also inhibit EGFR receptor (inhibiting autophagy by activating mTOR), which offset both the cellular signaling pathways against each other and result in no autophagy activation being observed. Thus it is also important to analyze the effect of drugs on the individual pathways to regulate autophagy. These profiles may lead to developing the combined use of different types of autophagy modulators such as lithium and rapamycin, which can gain the greater activation of autophagy (27). (Fig. 2).

Autophagy Modulation as a Potential Therapeutic Intervention for Lung Diseases

Although various classes of clinically used drug modulate autophagy pathways (Table 4), it is still unclear in many cases whether the therapeutic effects of those drugs arise from autophagy or autophagy-independent pathways. Even if autophagy is involved, there is still an important question remaining whether modulating autophagy by drugs contributes to their beneficial pharmacological effects. For example, metformin, one of the most commonly used drugs for treatment of Type 2 diabetes, induces autophagy (48, 70). The role of autophagy on the pathogenesis of diabetes is still controversial (94). Antidiabetic effects of metformin may be mediated by metformin-induced autophagy activation or conversely may be compromised by the autophagy activation. Recent clinical studies show new possible interventions using drugs for lung diseases including COPD (3) and tuberculosis (67). Azithromycin, a macrolide antibiotic, inhibits the frequency of exacerbation of COPD (3). High-dose supplement of vitamin D3 (VitD3) reduces time of sputum culture conversion of Mycobacterium tuberculosis (Mt) in patients with polymorphism of VitD3 receptor (67). Although the concise mechanisms by which these drugs exert the beneficial effects are still unclear, they commonly modulate autophagy activity. The pharmacological effect of azithromycin is similar with bafilomycin A1, a family of macrolide antibiotic and also a well-known autophagy inhibitor (96) (Fig. 1). The beneficial roles of azithromycin on COPD may be associated with the inhibitory effect of azithromycin on autophagy, since autophagy gene deficiency displays protective effects in a mouse model of COPD. In contrast, VitD3 inhibits replication of Mt by increasing autophagy activity (128). Interestingly, drugs can also increase autophagy activity in specific pathological condition. Isoniazid, an antibiotic for Mt, induces autophagy in macrophages infected with Mt, rather than in uninfected cells (49). Although further studies are needed to clarify how autophagy is involved in the beneficial effects of those drugs, these reports suggest that pharmacological manipulation on autophagy activity can serve as a potential therapeutic strategy in lung diseases. In addition, investigating the effect of drugs on autophagy activity can reveal the new mechanism of disease pathogenesis and previously unknown pharmacological actions of the drugs.

It is also important to explore the possibility to use autophagy modulators as an intervention for treatment of lung diseases. For example, an intervention to enhance autophagy activity may have beneficial effects in lung diseases such as tuberculosis or CF, since the effective drugs reported for those diseases activate autophagy (32, 67). However, the caution...
should be taken for this strategy. Autophagy has diverse effects depending on diseases or pathological conditions. Although activation of autophagy is beneficial for Mtb infection, the activated autophagy may also exert protective roles for microbes such as HIV or HBV (99). If patients with tuberculosis have latent infections with HIV or HBV, it is possible that autophagy activation promotes replication and survival of those microbes in the patients (99). On the other hand, autophagy inducers may have the additive beneficial effects in patients enrolled in clinical trials for treatment of tuberculosis, such as patients with Parkinson disease, where autophagy plays protective roles (99). Finally, therapeutic interventions by modulating autophagy can add to the current therapeutic strategies, rather than be used solely. For example, a recent clinical trial for lung cancer utilizes the combined interventions of erlotinib, a tyrosine kinase inhibitor, and chloroquine (125). The anticancer mechanism of erlotinib is likely to be autophagy independent (36, 57). Inhibiting autophagy by chloroquine enhances anticancer effect of erlotinib by minimizing prosurvival effect of erlotinib-mediated autophagy in tumor cells (36, 57, 120, 125). Modulating autophagy activity may enhance the beneficial roles of autophagy or minimize the adverse effect of autophagy on each disease. Furthermore, adding autophagy modulators may improve the current pharmacological effect of drugs used for the treatment.

Although clinical trials for investigating the therapeutic effects of autophagy modulators are most desirable to observe the usefulness of drugs, cohort studies to examine the effect of autophagy modulator on patients’ prognosis would be more accessible. For example, there are a number of patients with COPD who use autophagy modulators such as Ca2+ blockers, statin, or metformin for their medication; these drugs may influence patients’ quality of life, lung function, and frequency of acute exacerbation. In addition, these studies may help to identify drugs which are suitable for the clinical trials.

CONCLUSION

Until now more than 20 different classes of drug used for treatment have been identified as autophagy modulators (9, 129) (Table 4). Moreover, the new-class autophagy modulators with improved specificity and effectiveness have been developed for human disease indications as potential therapeutics (Table 5). Given the important roles of autophagy in various lung diseases (Fig. 3 and Tables 2 and 3), utilizing autophagy modulators may improve the therapeutic effects of the current interventions in various lung diseases. However, there are several important points to keep in mind. Although similar situations are observed in other medical interventions, effective and less-invasive methods to monitor autophagy activity for patients (e.g., biomarkers) are not currently available. Second, since autophagy is such a fundamental cellular process that affects various pathophysiological conditions, it is plausible that modulating autophagy causes other untoward effects that may be clinically deleterious. Finally, the autophagy modulators listed in Tables 4 and 5 may have off-target effects in addition to modulating autophagy. Nonetheless, recent clinical studies using autophagy modulators (3, 32, 67) and ongoing clinical trials (more than 15 active trials targeting autophagy) still suggest that autophagy modulators unravel new avenues in therapeutic interventions of various human diseases including lung diseases. Although further development of assays for autophagy activity and drugs with improved selectivity are needed, the day when we use autophagy-modifying drugs to treat lung disease may come sooner than later.

ACKNOWLEDGMENTS

The authors thank Jeffrey Adam Haspel for critical review of this manuscript.

GRANTS

This study is supported by NIH T32HL007633-27 (K. Nakahira) and NIH PO1 HL105339 (A. M. K. Choi) and R01 HL05530 (A. M. K. Choi).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

K.N. and A.M.K.C. conception and design of research; K.N. drafted manuscript; K.N. and A.M.K.C. edited and revised manuscript; K.N. and A.M.K.C. approved final version of manuscript.

REFERENCES


41. Kim JH, Lee HM, Shin DM, Kim W, Yun JM, Jang HS, Lee SH, Cha GH, Kim JM, Lee ZW, Shin SJ, Yoo H, Park YK, Park JB, Chung J, Yoshimori T, Jo EK. Host cell autophagy activated by antibiotics is...
1. Autophagy and the immune system.


