IN TIMES OF NUTRIENT OR OXYGEN scarcity, cells survive by eating and recycling part of themselves (73, 94). These cellular processes are collectively named “autophagy” (in Greek, “eating oneself”). Autophagy is a cellular self-degradation process in which cytosolic materials and organelles are sequestered and delivered to lysosome for degradation and recycling (73, 94). In addition to nutrient starvation, autophagy is induced by various physiological and pathological conditions. Importantly, autophagy is regulated in various human diseases such as cancer (120), metabolic diseases (e.g., obesity and Type 2 diabetes) (91), neurodegenerative diseases (e.g., Alzheimer’s disease and Parkinson disease) (122), and infectious diseases [e.g., human immunodeficiency virus (HIV) and tuberculosis] (54, 60). These reports suggest that autophagy can serve as a potential therapeutic target for human diseases. Here, we summarize the pathophysiological roles of autophagy in lung disease, and we explore the possibility that modulating autophagy as a therapeutic strategy in lung disease 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–Gso and cyclic AMP (cAMP)–phospholipase Cε (PLCe)–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 autophagy 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 diseases.

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 activating 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 Ca2+ can activate autophagy by inhibiting both class I PI3K and mTOR. In the mTOR-independent pathway, increase of intracellular cAMP level and Ca2+ can activate autophagy by inhibiting both class I PI3K and mTOR. In the mTOR-independent pathway, increase of intracellular cAMP level and Ca2+ can activate autophagy by inhibiting both class I PI3K and mTOR. In the mTOR-independent pathway, increase of intracellular cAMP level and Ca2+ can activate autophagy by inhibiting both class I PI3K and mTOR. In the mTOR-independent pathway, increase of intracellular cAMP level and Ca2+ can activate autophagy by inhibiting both class I PI3K and mTOR. In the mTOR-independent pathway, increase of intracellular cAMP level and Ca2+ can activate autophagy by inhibiting both class I PI3K and mTOR.
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 (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. LC3 deficiency promotes CSE-induced IL-8 secretion in HBEC. Autophagy inhibition by bafilomycin A enhances CSE-induced IL-8 secretion in HBEC.</td>
<td>15, 16, 47</td>
</tr>
<tr>
<td>IPF</td>
<td>Autophagy activation by rapamycin inhibits IL-17A-induced collagen production in lung epithelial cells. 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. 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>71, 112</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 increase angiogenesis in PAEC from fetal lambs with persistent pulmonary hypertension (PPH-PAEC). Beclin-1 knockdown promotes angiogenesis in PPH-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. LC3 deficiency promotes growth of <em>B. cepacia</em> and IL-1β secretion in macrophages. Autophagy activation by rapamycin inhibits growth of <em>B. cepacia</em> and IL-1β secretion in macrophages. Autophagy activation reduces bacterial burden of lung and inflammation in lung of CF mice after <em>B. cepacia</em> infection. Overexpression of SQSTM1/p62 inhibits intracellular survival of <em>B. cepacia</em> in macrophages. Deficiency of SQSTM1/p62 promotes intracellular survival of <em>B. cepacia</em> in macrophages.</td>
<td>65, 1</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Blocking chaperone-mediated autophagy by LAMP-2A depletion suppresses cell proliferation and increases cell death in lung cancer cells. Injection of lung cancer cells with LAMP-2A deficiency reduces tumor formation in xenograft mouse model. LC3B deficiency promotes TGF-β-induced fibroblast activation in vitro. LC3B and Beclin 1 deficiency inhibits CSE-induced cell death in pulmonary epithelial cells. LC3B deficiency promotes TGF-β-induced fibronectin and α-SMA expression in lung fibroblast.</td>
<td>53, 59</td>
</tr>
<tr>
<td>LAM</td>
<td>TSC2 knockdown increases autophagy-dependent cell survival in mouse embryonic fibroblasts. Autophagy inhibition by chloroquine inhibits xenograft tumor size in mice of TSC2-null xenograft tumor. LC3B and Beclin 1 deficiency promotes TGF-β-induced fibroblast activation in vitro. LC3B deficiency promotes TGF-β-induced fibroblast activation in vitro. LC3B and Beclin 1 deficiency inhibits CSE-induced cell death in pulmonary epithelial cells. LC3B and Beclin 1 deficiency promotes TGF-β-induced fibroblast activation in vitro. LC3B and Beclin 1 deficiency promotes TGF-β-induced fibroblast activation in vitro. LC3B and Beclin 1 deficiency promotes TGF-β-induced fibroblast activation in vitro.</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. Autophagy activation by rapamycin restores CLP-induced myocardial dysfunction in mice. LC3 overexpression ameliorates acute lung injury and survival in CLP-induced polymicrobial model of sepsis. Knockdown of VPS34 increases cell death and liver injury in CLP-induced polymicrobial models of sepsis. Autophagy inhibition suppresses releases of neutrophil extracellular trap (NET) induced by <em>E. coli</em> in human neutrophils.</td>
<td>79, 40, 64, 14, 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; PAEC, 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 is an important cellular process to regulate or contribute to the pathogenesis of lung diseases. Importantly, Fig. 3 demonstrates specificity of the pathophysiological functions of autophagy in each lung disease, resulting in either favorable or deleterious phenotype depending in the disease process.

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

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### Table 3. The functional role of autophagic proteins on pathogen(s) causing respiratory tract infection

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Role of Autophagy or Autophagic Proteins</th>
<th>References</th>
</tr>
</thead>
</table>
| Tuberculosis (M. tuberculosis) | • Autophagy induction by rapamycin or starvation inhibits M. tuberculosis survival in infected macrophages.  
  • IL-4 and IL-13 inhibit autophagy activation and autophagy-mediated killing of intracellular M. tuberculosis in macrophages.  
  • Autophagy activation by rapamycin enhances presentation of mycobacterial antigen in macrophages.  
  Subcutaneous injection of mycobacteria-infected dendritic cells pretreated with rapamycin enhances Th1 response and increases vaccine efficacy in mice.  
  • Autophagy induction by Vitamin D3 promotes killing of M. tuberculosis.  
  • Isoniazid-induced autophagy is associated with antimicrobial activity in M. tuberculosis-infected macrophages. | 34  
 37  
42  
128  
49 |
| Avian influenza A H5N1 | • Knockdown of Atg5 and TSC2 inhibits H5N1-induced cell death in lung epithelial cells.  
  Autophagy inhibition by 3-MA ameliorates acute lung injury and mice survival rate in mice infected with H5N1 | 109 |
| P. aeruginosa | • Autophagy induction by rapamycin promotes P. aeruginosa clearance and autophagy inhibition by 3-MA inhibits the bacterial clearances in macrophages.  
  Knockdown of Beclin 1 inhibits P. aeruginosa clearance in macrophages. | 127 |
| M. abscessus | • Autophagy induction by Azithromycin inhibits intracellular killing of M. abscessus in macrophages.  
  Autophagy induction by Azithromycin promotes M. abscessus infection in mice. | 96 |
| Human rhinovirus 2 | • Autophagy activation by rapamycin increases replication of human rhinovirus 2 (HRV-2).  
  • Autophagy inhibition by 3-MA suppresses replication of HRV-2. | 50 |
| Human adenovirus type 5 | • Knockdown of Atg5 inhibits human adenovirus-mediated cell lysis. | 44 |
| S. aureus | • Knockdown of Atg5 inhibits replication of S. aureus and S. aureus-mediated cell death. | 105 |
| Respiratory syncytial virus | • Knockdown of Beclin 1 or LC3 attenuates respiratory syncytial virus-induced proinflammatory cytokines production. | 78 |

*M. tuberculosis, Mycobacterium tuberculosis; P. aeruginosa, Pseudomonas aeruginosa; M. abscessus, Mycobacterium abscessus; S. aureus, Staphylococcus aureus.*
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 molecules modulating autophagy activity. There are several target signaling pathways wherein autophagy activity is modulated by drugs.

**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>
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</thead>
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<tr>
<td>Autophagy inducers</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 phytoalexins</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>
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<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 Ca²⁺ channel blockers)</td>
<td>Vasodilator</td>
<td>Reduce intracellular Ca²⁺ levels</td>
<td>9, 121, 129</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>Anticonvulsant</td>
<td>Inhibits histone deacetylase and reduces intracellular Ca²⁺ 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>
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<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>
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<tr>
<td>Statin (HMG-CoA reductase inhibitor)</td>
<td>Lower cholesterol</td>
<td>Depletes geranylgeranyl diphosphate</td>
<td>83</td>
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<tr>
<td>Vitamin D3</td>
<td>Supplementary diet to reduce the risk of fractures and falls</td>
<td>Increases Beclin 1 expression by upregulating cathelicidin</td>
<td>128</td>
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<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 antipapoptotic proteins BCL-2</td>
<td>66</td>
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<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>Vasodilator</td>
<td>Unknown</td>
<td>121</td>
</tr>
<tr>
<td>Salbutamol (β₂ adrenergic receptor agonist)</td>
<td>Treatment for asthma by smooth muscle relaxant</td>
<td>Unknown</td>
<td>6</td>
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<tr>
<td>Isoniazid, pyrazinamide</td>
<td>Antibiotics for tuberculosis</td>
<td>AMPK and intracellular Ca²⁺ dependent</td>
<td>49</td>
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<tr>
<td>Autophagy inhibitors</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Macrolide antibiotic</td>
<td>Inhibits fusion of autophagosome and lysosome</td>
<td>96</td>
</tr>
<tr>
<td>Chloroquine/hydroxychloroquine</td>
<td>Treatment for RA, SLE, and malaria Undergoing clinical trials for various cancers</td>
<td>Inhibits fusion of autophagosome and lysosome</td>
<td>4, 12</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>Anticancer</td>
<td>Inhibits microtubule formation</td>
<td>89</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>
<tr>
<td>IL-13</td>
<td>Undergoing clinical trial for advanced cancers</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\textsuperscript{2+} activate mTOR and lead to inhibition of autophagy (27). Lithium also inhibits GSK-3β, which has been shown to activate autophagy via an mTOR-independent pathway. Although lithium is possible that some drugs can stimulate both proautophagy and antiautophagy pathways. For example, although lithium is inhibitory to GSK-3β, which can activate mTOR and lead to inhibition of autophagy (27). In addition, some drugs may inhibit Ca\textsuperscript{2+} channel (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).

**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* (Mbt) 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 Mbt by increasing autophagy activity (128). Interestingly, drugs can also increase autophagy activity in specific pathological condition. Isoniazid, an antibiotic for Mbt, induces autophagy in macrophages infected with Mbt, 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.

**Table 5. Molecules or compounds that modulate autophagy activity**

<table>
<thead>
<tr>
<th>Molecules or Compounds</th>
<th>Mechanism of Action in Autophagy Pathway</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autophagy activators</strong></td>
<td>Torin 1</td>
<td>Directly inhibits both mTORC1 and mTORC2</td>
</tr>
<tr>
<td></td>
<td>PP242</td>
<td>Inhibits mTORC1</td>
</tr>
<tr>
<td></td>
<td>PI103 hydrochloride</td>
<td>Highly selective class I PI3K inhibitor and ATP-competitive mTOR inhibitor</td>
</tr>
<tr>
<td></td>
<td>2'-5'-Dideoxyadenosine</td>
<td>Reduces cAMP levels (Adenylyl cyclase inhibitor)</td>
</tr>
<tr>
<td></td>
<td>Xestospongin B</td>
<td>IP3R antagonist</td>
</tr>
<tr>
<td></td>
<td>Sperrmine</td>
<td>Postulated to affect expression of Atg genes</td>
</tr>
<tr>
<td></td>
<td>Tat-beclin1</td>
<td>A cell permeable peptide derived from a region (267–284) of Beclin 1</td>
</tr>
<tr>
<td></td>
<td>Calpastatin, calpeptin</td>
<td>Inhibit calpain</td>
</tr>
<tr>
<td></td>
<td>Trehalose (an α-linked disaccharide)</td>
<td>mTOR independent</td>
</tr>
<tr>
<td></td>
<td>1'-NAME (inhibition of NOS and decrease of NO production)</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>GGTI-298 (inhibition of geranylgeranyl transferase 1)</td>
<td>P53 dependent</td>
</tr>
<tr>
<td><strong>Autophagy inhibitors</strong></td>
<td>Spautin-1</td>
<td>Lowers Beclin 1 levels by promoting its ubiquitination</td>
</tr>
<tr>
<td></td>
<td>3-MA</td>
<td>Inhibits class III PI3K</td>
</tr>
<tr>
<td></td>
<td>Pepstatin A</td>
<td>Inhibits aspartyl protease (cathepsin D)</td>
</tr>
<tr>
<td></td>
<td>Cystatin B</td>
<td>Inhibits cystatine protease (cathepsin B)</td>
</tr>
<tr>
<td></td>
<td>Leupeptin</td>
<td>Inhibits serine and cysteine proteases</td>
</tr>
<tr>
<td></td>
<td>Bafilomycin A1</td>
<td>Inhibits V-ATPase; Inhibits fusion of autophagosome and lysosome</td>
</tr>
<tr>
<td></td>
<td>Nocodazole</td>
<td>Inhibits microtubule formation</td>
</tr>
</tbody>
</table>

IP3R, inositol-(1,4,5)-trisphosphate receptor; L-NAME, N'-l-arginine methyl ester; 3-MA, 3-methyladenine; NO, nitric oxide; NOS, nitric oxide synthase; Atg, autophagy-related; V-ATPase, vacuolar ATPase.
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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 drug 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 also 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. Secondly, 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.

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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.

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