Autophagy: a potential therapeutic target in lung diseases

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Running title:

Autophagy modulation in lung diseases
Abstract:

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 in not only 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.
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 that 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 II diabetes) (91), neurodegenerative diseases (e.g. Alzheimer’s disease and Parkinson disease) (122) and infectious diseases (e.g. HIV and tuberculosis)(54, 60). These reports suggest autophagy can serve as a potential therapeutic target for human diseases. Here, we summarize the patho-physiological roles of autophagy in lung disease, and explore the possibility that modulating autophagy activity using pharmacological approach can be a useful therapeutic intervention.

**Molecular mechanism of Autophagy**

Autophagy encloses cytosolic materials by isolation membranes (phagophores) to form double membrane-bound vesicles called ‘autophagosomes’ (Figure 1). The isolation membranes are acquired from multiple sources including endoplasmic reticulum (ER), 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.
More than 30 autophagy related genes (Atg) proteins have been identified to be involved in autophagosome formation (23, 30). 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 (Figure 2, left panel). In mTOR-independent pathways, Ca$^{2+}$–calpain–Gs$\alpha$ and cyclic AMP (cAMP)–Phospholipase C epsilon (PLC$\varepsilon$)–Inositol-(1,4,5)-trisphosphate (IP3) pathways are involved in autophagy activation (Figure 2, right panel).

**Function of Autophagy**

While autophagy non-selectively 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 physiologic 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. While 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 pyrogen (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 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 such as bacterial agent such as Brucella abortus (99) and Coxiella burnetii (99), viruses such as HIV (99), HBV (54, 99) and avian influenza A H5N1(H5N1) (109). Of note, autophagy exerts both killing and pro-survival 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 multi-protein 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 (MHC) 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 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 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 TCA cycle-related metabolites (33), suggesting 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 such as Parkinson disease (68, 80, 81, 118). Thus, 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. have recently reported the guideline for monitoring autophagy in higher eukaryotes and described useful methods and how to interpret the data (51). 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 autophagosome 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 state), rather than relying on the results from a single method (51, 101).
**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 based on studies using genetic or biochemical perturbation of autophagy. Although autophagy has been initially thought as a cytoprotective response in patho-physiological 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 to the control mice (16). Similar adverse effects of autophagy are observed in other lung diseases including lung cancer (36, 46, 53) and acute lung injury induced by H5N1 infection (109). In contrast, the beneficial roles of autophagy are also observed in the diseases such as cystic fibrosis (CF) (1, 2, 65), tuberculosis (23, 34, 42) and sepsis (14, 40, 45, 64, 79). Thus,
autophagy is an important cellular process to regulate or contribute to the pathogenesis of lung diseases. Importantly, Figure 3 demonstrates specificity of the patho-physiologic 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 (SCLC) 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 (NSCLC) in two clinical trials (Phase 1/2 and 2) (125). Although autophagy has been initially shown to be associated with anti-tumorgenesis 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). While initiation of tumorigenesis in normal cells is associated with defect of autophagy, autophagy subsequently may exert pro-survival 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 unlike H1N1, where genetic and biochemical inhibition of autophagy rescue 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 autophagy modulation by clinically-used drugs may be more accessible to study in clinical trials due to their favorable safety profiles. The details of ongoing clinical trial targeting modulating autophagy are listed (http://www.clinicaltrial.gov/ct2/results?term=autophagy&Search=Search).

Autophagy modulators
Given the important association between autophagy and human lung diseases, it is worthwhile to review if modulating autophagy can serve as potent therapeutic targets for various lung diseases. Table 4 demonstrates a list of autophagy modulating drugs which 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.

(a) mTOR signaling pathways (Figure 2, left panel)

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). Rapamycin and its analogues 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 autophagy greater than rapamycin (115). Of note, another new class drugs have 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).

(b) mTOR-independent pathways (Figure 2, right panel)

• 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.
Intracellular Ca\(^{2+}\) and cAMP negatively regulate autophagy by increasing intracellular inositol level (94). In addition, calpain activated by elevated level of intracellular Ca\(^{2+}\) inhibits autophagy by cleavage of Atg5 (94, 99). Antihypertensive drugs including Ca\(^{2+}\) receptor blockers (9, 121, 129) or imidazolin receptor agonists (98, 121) induce autophagy by decreasing cAMP.

(c) Degradation steps of autophagy (Figure 1)

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

(d) 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) (Figure 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-NAME (104), trehalose (102) or Beta2 adnergic receptor agonist (6) whose mechanisms to modulate autophagy are still unclear (Table 4). Further studies will be needed to identify concise pathways by which those drugs or compounds modulate autophagy.

Since there are several pathways regulating autophagy, it is possible that some drugs can stimulate both pro-autophagy and anti-autophagy pathways. For example, although lithium has been shown to activate autophagy via mTOR
independent pathway, lithium also inhibits GSK-3β which can activate mTOR and lead to inhibition of autophagy (27) (Figure 2). In addition, some drugs may inhibit Ca²⁺ channel (activating autophagy by mTOR independent pathways) and also inhibit EGFR receptor (inhibiting autophagy by activating mTOR), which offset the both cellular signaling pathways each other and result in no autophagy activation 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) (Figure 2).

Autophagy modulation as a potential therapeutic intervention for lung diseases. While 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 if modulating autophagy by drugs contributes to their beneficial pharmacological effects. For example, metformin, one of the most commonly used drugs for treatment of type II diabetes, induces autophagy (48, 70). The role of autophagy on the pathogenesis of the 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). While 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) (Figure 1). The beneficial roles of azithromycin on COPD may be associated with the inhibitory effect of azithromycin on autophagy, as 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.

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, as 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. While activation of autophagy is beneficial for Mbt 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 hands, autophagy inducers may have the additive beneficial effects in patients enrolled clinical trials for treatment of tuberculosis, such as patients have Parkinson diseases, 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 examples, a recent clinical trial for lung cancer utilizes the combined interventions of erlotinib, a tyrosine kinase inhibitor, and chloroquine (125). Anti-cancer mechanism of erlotinib is likely to be autophagy-independent (36, 57). Inhibiting autophagy by chloroquine enhances anti-cancer effect of erlotinib by minimizing pro-survival 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.
While 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 Ca\(^{2+}\) 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 twenty 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 (Figure 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 patho-physiological conditions, it is plausible that modulating autophagy causes other untoward effects that may be are 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 of 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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Figure legends

Figure 1. Autophagy process and potential targets for modulating autophagy. Activation of UNC51-like kinase (ULK) complex in response to certain signals initiates isolation membrane and the formation of phagophore. (Upstream pathway of ULK1 complex is depicted in Figure 2.) Class III PI3K complex composed of Beclin 1, class III PI3K, PIK3R4 and activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1) is also required for the phagophore formation. The Atg-Atg12-Atg16L complex and LC3-II phosphatidylethanolamine (PE) conjugate promote the elongation and enwrap the cytosolic cargos including mitochondria, leading to the formation of autophagosome. Subsequently, lysosome fuses with the autophagosome (the
formation of autolysosome) and release acid hydrases into the interiors to
degradate the cytosolic cargos.

Autophagy can be activated by drugs such as BCL-2 homology 3 (BH3) mimetics
which prevent the formation of autophagy inhibitory complex of Beclin 1 and
BCL-2. In contrast, Ubiquitin-mediated decrease of Beclin 1 protein by Spautin-1
and inhibition of class III PI3K by 3-methyladenine (3-MA) can inhibit autophagy.

In addition, there are drugs to inhibit the late phase of autophagy process.

Lysosomotropic agents that enhance lysosomal ph such as chloroquine inhibit
lysosomal enzymes and also prevent the fusion of autophagosome and
lysosome, resulting in inhibition of autophagy. Bafilomycin A1 inhibits the fusion
of autophagosome and lysosome by inhibiting vacuolar ATPase (V-ATPase)
located in the lysosomal membrane. Compounds such as pepstain A and
cystatin B are inhibitors for lysosomal protease. (Please also see Table 4).

Figure 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: 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 mTOR-independent pathway, increase of intracellular cAMP level and Ca\(^{2+}\) inhibits autophagy. Intracellular level of cAMP is upregulated by adenylyl cyclase (AC) and calpain-Gs\(\alpha\) pathway which is activated by intracellular Ca\(^{2+}\) level. The inhibitory effect of cAMP on autophagy is mediated by increase of synthesis of IP3 and inositol. Increased level of IP3 activates IP3 receptor in ER and release Ca\(^{2+}\) into cytosol, leading to inhibition of autophagy. Activation of L-type Ca\(^{2+}\) channel triggers Ca\(^{2+}\) influx and elevates intracellular Ca\(^{2+}\) level, which activates cysteine protease called calpain. In addition to elevating cAMP by calpain-Gs\(\alpha\) pathway, calpain inhibits autophagy by cleavage of Atg5. However, the concise mechanism by which cytosolic cAMP, Ca\(^{2+}\), inositol and IP3 regulate autophagy is still unclear. In this cyclical mTOR independent pathway, autophagy can be modulated by mainly targeting intracellular levels of cAMP or Ca\(^{2+}\). L-type Ca\(^{2+}\) receptor blockers such as verapamil inhibit Ca\(^{2+}\) 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\(^{2+}\) release by IP3 receptor (IP3R).

Abbreviation in Figure 2: EGFR, epidermal growth factor receptor; PI3K, phosphoinositide 3-kinase; IGF, Insulin-like growth; TSC, tuberous sclerosis 2; ULK1, UNC51-like kinase; AMPK, AMP-activated protein kinase; V-ATPase, vacuolar ATPase; I1R, imidazoline-1 receptor; PLC\(\varepsilon\), phospholipase C epsilon; ER, endoplasmic reticulum; 2′,5′-dideoxyadenosine; PIP2, phosphatidylinositol-4,5-bisphosphate; cAMP, cyclic AMP; Ins, inositol; IP,
Figure 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 tumorgenesis, while autophagy can promote survival and proliferation of tumor cells. In infectious diseases, the roles of autophagy are dependent on microbes. Autophagy may promotes bacterial-killing and inhibit intracellular survival of microbes such as *M. tuberculosis*. On the other hands, autophagy may promote replication and survival of microbes such as H5N1.
### Table 1: Methods for monitoring autophagy *in vitro* and *in vivo*.

<table>
<thead>
<tr>
<th>Monitoring state of autophagy</th>
<th>Methods</th>
<th>Key points</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Monitoring static state</td>
<td></td>
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<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.</td>
<td>(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.</td>
<td>(75)</td>
</tr>
</tbody>
</table>
| Puncta formation of LC3      | Immunofluorescence microscopy | • This method can be applied to *in vivo* using GFP-LC3 transgenic mice.  
• Increased puncta formation of LC3 does not reliably correlate with autophagic flux.                                                                                           | (72, 74) |
| Monitoring dynamic state     |                          |                                                                                                                                                                                                                                                                                                                                         |          |
| Turnover of LC3-II           | Western blot            | • 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) |
| Turnover of p62/SQSTM1       | Western blot            | • 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) |
| Cytosolic protein sequestration assays | Western blot | • 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) |
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>Refs.</th>
</tr>
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<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. &lt;br&gt; • 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) (28)</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 antagonist on bleomycin-induced lung fibrosis and mortality of mice. &lt;br&gt; • 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. &lt;br&gt; • Rapamycin inhibits anti-TLR4 antibody-induced lung fibrosis in mice. &lt;br&gt; • Atg5 and LC3 deficiency promotes TGF-β induced fibroblast activation in vitro. Inhibition of mTOR by Torin1 inhibits tunicamycin (ER stress inducer)-induced senescence in HBEC. Atg5 and LC3 deficiency promotes tunicamycin-induced senescence in HBEC.</td>
<td>(71) (85) (124) (7)</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. &lt;br&gt; • Autophagy inhibition by 3-MA and chloroquine increase angiogenesis in PAEC from fetal lambs with persistent pulmonary hypertension (PPHN-PAEC). &lt;br&gt; • 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. &lt;br&gt; • 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. &lt;br&gt; • 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>(65) (1) (2)</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. &lt;br&gt; • 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. &lt;br&gt; • Atg7 deficiency reduces cancer cell proliferation in NSCLC cell lines. Atg7 deficiency sensitizes the cancer cells to cisplatin-induced apoptosis in NSCLC cell lines. &lt;br&gt; • Atg3 deficiency reverses erlotinib resistance in erlotinib-resistant lung cancer cells.</td>
<td>(53) (36) (46) (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. &lt;br&gt; • Autophagy activation by rapamycin restores CLP-induced myocardial dysfunction in mice. &lt;br&gt; • LC3 overexpression ameliorates acute lung injury and survival in CLP-induced polymicrobial model of sepsis.</td>
<td>(79) (40) (64)</td>
</tr>
</tbody>
</table>
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 E. Coli in human neutrophils.


**Abbreviations:** 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; PAH, pulmonary (artery) hypertension; PAEC, pulmonary artery endothelial cells; CF, cystic fibrosis; MPO, myeloperoxidase; B. cepacia, Burkholderia cenocepacia; NSCLC, non-small 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.
### 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>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tuberculosis</strong> (&lt;i&gt;M. tuberculosis&lt;/i&gt;)</td>
<td>• Autophagy induction by rapamycin or starvation inhibits &lt;i&gt;M. tuberculosis&lt;/i&gt; survival in infected macrophages. &lt;br&gt;• IL-4 and IL-13 inhibit autophagy activation and autophagy-mediated killing of intracellular &lt;i&gt;M. tuberculosis&lt;/i&gt; in macrophages. &lt;br&gt;• 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. &lt;br&gt;• Autophagy induction by Vitamin D3 promotes killing of &lt;i&gt;M. tuberculosis&lt;/i&gt;. &lt;br&gt;• Isoniazid-induced autophagy is associated with antimicrobial activity in &lt;i&gt;M. tuberculosis&lt;/i&gt;-infected macrophages.</td>
<td>(34) (37) (42) (128) (49)</td>
</tr>
<tr>
<td>Avian influenza A H5N1</td>
<td>• 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 Knockdown of Atg5 ameliorates acute lung injury and mice survival rate in mice infected with H5N1</td>
<td>(109)</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>• Autophagy induction by rapamycin promotes &lt;i&gt;P. aeruginosa&lt;/i&gt; clearance and autophagy inhibition by 3-MA inhibits the bacterial clearances in macrophages Knockdown of Beclin 1 inhibits &lt;i&gt;P. aeruginosa&lt;/i&gt; clearance in macrophages.</td>
<td>(127)</td>
</tr>
<tr>
<td><strong>M. abscessus</strong></td>
<td>• Autophagy inhibition by Azithromycin inhibits intracellular killing of &lt;i&gt;M. abscessus&lt;/i&gt; in macrophages. Autophagy inhibition by Azithromycin promotes &lt;i&gt;M. abscessus&lt;/i&gt; infection in mice.</td>
<td>(96)</td>
</tr>
<tr>
<td>Human Rhinovirus 2</td>
<td>• Autophagy activation by rapamycin increases replication of human Rhinovirus 2 (HRV-2)</td>
<td>(50)</td>
</tr>
<tr>
<td>Human Adenovirus type 5</td>
<td>• Autophagy inhibition by 3-MA suppresses replication of HRV-2</td>
<td></td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>• Knockdown of Atg5 inhibits replication of &lt;i&gt;S. aureus&lt;/i&gt; and &lt;i&gt;S. aureus&lt;/i&gt;-mediated cell death.</td>
<td>(105)</td>
</tr>
<tr>
<td><strong>Respiratory Syncytial virus</strong></td>
<td>• Knockdown of Beclin 1 or LC3 attenuates respiratory syncytial virus-induced proinflammatory cytokines production.</td>
<td>(78)</td>
</tr>
</tbody>
</table>


---

24
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>Refs.</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>Immunosupressant 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 (eritinib 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>Anti-cutaneous 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 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>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Antiepileptic</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 cathelicidin</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 anti-apoptotic proteins BCL-2</td>
<td>(66)</td>
</tr>
<tr>
<td>Carbon monoxide (at 250 p.p.m)</td>
<td>Vasodilator, anti-inflammation and anti-proliferation</td>
<td>Undergoing clinical trials for idiopathic pulmonary fibrosis and Sepsis</td>
<td>Increases mitochondrial reactive oxygen species.</td>
</tr>
<tr>
<td>Minoxidil (Potassium channel opener)</td>
<td>Vasodilator</td>
<td>Unknown</td>
<td>(121)</td>
</tr>
<tr>
<td>Salbutamol (Beta2 adnergic receptor agonist)</td>
<td>Treatment for asthma by smooth muscle relaxant.</td>
<td>Unknown</td>
<td>(6)</td>
</tr>
<tr>
<td>Isoniazid, Pyrazinamide</td>
<td>Antibiotics for tuberculosis</td>
<td>AMPK and intracellular Ca²⁺ 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>(96)</td>
</tr>
<tr>
<td>Chloroquine/Hydroxychloroquine</td>
<td>Treatment for RA, SLE and Malaria</td>
<td>Undergoing clinical trials for various cancers</td>
<td>(4, 12)</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>Anti-cancer</td>
<td>Inhibits fusion of autophagosome and lysosome</td>
<td>(4, 12)</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>

**Abbreviation:** mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; PI3K, phosphoinositide 3-kinase; NF-xB, nuclear factor-kappa B, 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; BCL-2, 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.clinicaltrial.gov/](http://www.clinicaltrial.gov/).

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>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autophagy activators</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torin1</td>
<td>Directly inhibits both mTORC1 and mTORC2</td>
<td>(115)</td>
</tr>
<tr>
<td>PP242</td>
<td>Inhibits mTORC1</td>
<td>(26)</td>
</tr>
<tr>
<td>PI103 hydrochloride</td>
<td>Highly selective class I PI3K inhibitor and ATP-competitive mTOR inhibitor</td>
<td>(22)</td>
</tr>
<tr>
<td>2',5'-Dideoxyadenosine</td>
<td>Reduces cAMP levels (Adenynyl cyclase inhibitor)</td>
<td>(121)</td>
</tr>
<tr>
<td>Xestospongin B</td>
<td>IP3R antagonist</td>
<td>(117)</td>
</tr>
<tr>
<td>Spermidine</td>
<td>Postulated to affect expression of Atg genes</td>
<td>(24)</td>
</tr>
<tr>
<td>Tat-beclin1</td>
<td>A cell permeable peptide derived from a region (267-284) of Beclin 1</td>
<td>(107)</td>
</tr>
<tr>
<td>Calpastatin, calpeptin</td>
<td>Inhibit calpain</td>
<td>(121)</td>
</tr>
<tr>
<td>Trehalose (an alpha-linked disaccharide)</td>
<td>mTOR-independent</td>
<td>(102)</td>
</tr>
<tr>
<td>L-NAME (Inhibition of NOS and decrease of NO production)</td>
<td>Unknown</td>
<td>(104)</td>
</tr>
<tr>
<td>GGti-298 (Inhibition of Geranylgeranyl transferase 1)</td>
<td>P53-dependent</td>
<td>(29)</td>
</tr>
<tr>
<td><strong>Autophagy inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spautin-1</td>
<td>Lowers Beclin 1 levels by promoting its ubiquitination.</td>
<td>(63)</td>
</tr>
<tr>
<td>3-MA</td>
<td>Inhibits class III PI3K</td>
<td>(100)</td>
</tr>
<tr>
<td>Pepstatin A</td>
<td>Inhibits aspartyl protease (cathepsin D)</td>
<td>(113)</td>
</tr>
<tr>
<td>Cystatin B</td>
<td>Inhibits cystatine protease (cathepsin B)</td>
<td>(99)</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>Inhibits serine and cysteine proteases</td>
<td>(38)</td>
</tr>
<tr>
<td>Bafilomycin A1</td>
<td>Inhibits V-ATPase. Inhibits fusion of autophagosome and lysosome</td>
<td>(92, 123)</td>
</tr>
<tr>
<td>Nocodazole</td>
<td>Inhibit microtubule formation</td>
<td>(97, 119)</td>
</tr>
</tbody>
</table>

**Abbreviation:** 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.
References:


Gorzalczy Y, Gilad Y, Amihai D, Hammel I, Sagi-Eisenberg R, and Merimsky O. Combining an EGFR directed tyrosine kinase inhibitor with autophagy-


CT, Chuang TH, Chueh SH, Chun T, Chwae YJ, Chye ML, Ciarcia R, Ciriolo MR,
Clague MJ, Clark RS, Clarke PG, Clarke R, Codogno P, Coller HA, Colombo MI,
Comincini S, Condello M, Condorelli F, Cookson MR, Coombs GH, Coppens I,
Corbalan R, Cossart P, Costelli P, Costes S, Coto-Montes A, Couve E, Coxon FP,
Cregg JM, Crespo JL, Cronje MJ, Cuervo AM, Cullen JJ, Czaja MJ, D'Amelio M,
Darfeuille-Michaud A, Davids LM, Davies FE, De Felici M, de Groot JF, de Haan
CA, De Martino L, De Milito A, De Tata V, Debnath J, Degterev A, Dehay B,
SD, De Venish RJ, Di Gioacchino M, Di Paolo G, Di Pietro C, Diaz-Araya G, Diaz-
Laviada I, Diaz-Meco MT, Diaz-Nido J, Dikic I, Dinesh-Kumar SP, Ding WX,
Distelhorst CW, Diwan A, Djavaheri-Mergny M, Dokudovskaya S, Dong Z, Dorsey
FC, Dosenko V, Dowling JJ, Doxsey S, Dreux M, Drew ME, Duan Q, Duchosal MA,
Duff K, Dugail I, Durbeej M, Duschenko M, Edelstein CL, Edinger AL, Egea G,
Eichinger L, Eissa NT, Ekmekcioglu S, El-Deiry WS, Elazar Z, Elgendy M, Ellerby
Y, Fanto M, Fanzani A, Farkas T, Farre JC, Faure M, Fechheimer M, Feng CG,
Feng J, Feng Q, Feng Y, Fesus L, Feuer R, Figueiredo-Pereira ME, Fimia GM,
Fingar DC, Finkbeiner S, Finkel T, Finley KD, Fiorito F, Fisher EA, Fisher PB,
R, Fowler DH, Fox HS, Franco R, Frankel LB, Fransen M, Fuentes JM, Fueyo J,
Fujii J, Fujisaki K, Fujita E, Fukuda M, Furukawa RH, Gaestel M, Gaillly P,
Gajewska M, Galliot B, Galy V, Ganesh S, Ganetzky B, Ganley IG, Gao FB, Gao
Opota O, Osborne TF, Ostrander GK, Otsu K, Ou JH, Ouimet M, Overholtzer M,
Ozpolar B, Paganetti P, Pagnini U, Pallet N, Palmer GE, Palumbo C, Pan T,
Panaretakis T, Pandey UB, Papackova Z, Papassideri I, Paris I, Park J, Park OK,
Parys JB, Parzych KR, Patschan S, Patterson C, Pattingre S, Pawelek JM, Peng J,
Perlmutter DH, Perrotta I, Perry G, Pervaiz S, Peter M, Peters GJ, Petersen M,
Petrovski G, Phang JM, Piacentini M, Pierre P, Pierrefite-Carle V, Pierron G,
Pous C, Pozuelo-Rubio M, Praetorius-Ibba M, Prasad A, Prescott M, Priault M,
Produit-Zengaffinen N, Progulske-Fox A, Proikas-Cezanne T, Przedborski S,
Przyklenk K, Puertollano R, Puyal J, Qian SB, Qin L, Qin ZH, Quaggin SE, Raben
N, Rabinowich H, Rabkin SW, Rahman I, Rami A, Ramm G, Randall G, Randow F,
Rao VA, Rathmell JC, Ravikumar B, Ray SK, Reed BH, Reed JC, Reggiori F,
Regnier-Vigouroux A, Reichert AS, Reiners JJ, Jr., Reiter RJ, Ren J, Revuelta JL,
Rhodes CJ, Ritis K, Rizzo E, Robbins J, Roberge M, Roca H, Roccheri MC, Rocchi
S, Rodemann HP, Rodriguez de Cordoba S, Rohrer B, Roninson IB, Rosen K, Rost-
Roszkowska MM, Rouis M, Rouschop KM, Rotveta F, Rubin BP, Rubinsztein DC,
Ruckdeschel K, Rucker EB, 3rd, Rudich A, Rudolf E, Ruiz-Opazo N, Russo R,
Rusten TE, Ryan KM, Ryter SW, Sabatini DM, Sadoshima J, Saha T, Saitoh T,
Sakagami H, Sakai Y, Salekdeh GH, Salomoni P, Salvaterra PM, Salvesen G,
Salvioli R, Sanchez AM, Sanchez-Alcazar JA, Sanchez-Prieto R, Sandri M, Sankar
U, Sansanwal P, Santambrogio L, Saran S, Sarkar S, Sarwal M, Sasakawa C,
Sasnauskiene A, Sass M, Sato K, Sato M, Schapira AH, Scharl M, Schatzl HM,
Scheper W, Schiaffino S, Schneider C, Schneider ME, Schneider-Stock R,
Schoenlein PV, Schorderet DF, Schuller C, Schwartz GK, Scorrano L, Sealy L,
Seglen PO, Segura-Aguilar J, Seiliez I, Seleverstov O, Sell C, Seo JB, Separovic D,
Setaluri V, Setoguchi T, Settembre C, Shacka JJ, Shanmugam M, Shapiro IM,
Shaulian E, Shaw RJ, Shelhamer JH, Shen HM, Shen WC, Sheng ZH, Shi Y,
Shibuya K, Shidoji Y, Shieh JJ, Shih CM, Shimada Y, Shimizu S, Shintani T,
Shirihai OS, Shore GC, Sibirny AA, Sidhu SB, Sikorska B, Silva-Zacarin EC,
Simmons A, Simon AK, Simon HU, Simone C, Simonsen A, Sinclair DA, Singh R,
Sinha D, Sinicrope FA, Sirko A, Siu PM, Sivridis E, Skop V, Skulachev VP, Slack
RS, Smaili SS, Smith DR, Soengas MS, Soldati T, Song X, Sood AK, Soong TW,
Sotgia F, Specter SA, Spies CD, Springer W, Srinivasula SM, Stefanis L, Steffan JS,
Subauste CS, Sui X, Sulzer D, Sun J, Sun SY, Sun ZJ, Sung JJ, Suzuki K, Suzuki T,
Swanson MS, Swanton C, Sweeney ST, Sy LK, Szabadkai G, Tabas I, Taegtmeyer H,
Tafani M, Takacs-Vellai K, Takano Y, Takegawa K, Takemura G, Takeshita F,
Talbot NJ, Tan KS, Tanaka K, Tang D, Tanida I, Tannous BA, Tavernarakis N,
K, Thompson CB, Thorburn A, Thumm M, Tian F, Tian Y, Tocchini-Valentini G,
Tolkovsky AM, Tomino Y, Tonges L, Tooze SA, Tournier C, Tower J, Towns R,
Trajkovic V, Travassos LH, Tsai TF, Tschan MP, Tsubata T, Tsung A, Turk B,
Turner LS, Tyagi SC, Uchiyama Y, Ueno T, Umekawa M, Umemiya-Shirafuji R,
Unni VK, Vaccaro MI, Valente EM, Van den Berghe G, van der Klei IJ, van Doorn
W, van Dyk LF, van Egmond M, van Grunsven LA, Vandenabeele P,
Vandenberghe WP, Vanhorebeek I, Vaquero EC, Velasco G, Vellai T, Vicencio JM,


Mi S, Li Z, Yang HZ, Liu H, Wang JP, Ma YG, Wang XX, Liu HZ, Sun W, and Hu ZW. Blocking IL-17A promotes the resolution of pulmonary inflammation and


79. **Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, Fitzgerald KA, Ryter SW, and Choi AM.** Autophagy proteins regulate innate immune responses by inhibiting the release of


Figure 1

Initiation, isolation membrane

Elongation

Completion

Autophagosome and Lysosome fusion

Degradation

Autophagy activator

Autophagy inhibitor

LC3 II

Class III PI3K complex

Atg5-Atg12-Atg16L

Class III PI3K complex

Phagophore

Autophagosome

Autolysosome

Lysosome

Chloroquine

Bafilomycin A

Azithromycin

Pepstatin A

Cystatin B

3-MA

BCL-2

BH3 mimetics

Spautin 1

ULK1 complex

Beclin 1

Class III PI3K
Figure 2

- EGFR antagonists (ertinib)
- Metformin
- Rapamycin and analogues, Torin 1

Autophagy inhibitor: Rapamycin and analogues, Torin 1

Autophagy activator: EGFR antagonists (ertinib), Metformin

- Cell membrane
- Cytoplasm
- Receptor tyrosine kinase
- Class I PI3K
- Akt
- TSC1/2
- Rheb
- mTOR
- ULK1 complex
- Ca\(^{2+}\) channel
- cAMP
- Epac
- Rap2B
- PLC-\(\gamma\)72
- IP2
- IP1
- IMPase
- Ins
- I1R
- Gs\(\alpha\)
- ATP
- 2'5'ddA
- I1R
- AC
- PLC-\(\gamma\)72
- PIP2
- Ca\(^{2+}\) channel blockers (verapamil, amidarone)
- Calpastatin, calpeptin
- Calpain

Autophagy inhibitor: Rapamycin and analogues, Torin 1

Autophagy activator: EGFR antagonists (ertinib), Metformin

Autophagy activator: Lithium, L-960,330, Valproic acid, Carbamazepine

via cleavage of Atg5
Figure 3

- **LAM**: Promoting survival and proliferation of LAM cells
- **Cystic fibrosis**: Clearance of protein aggregate and regulating oxidative stress
- **Lung infection**: Promoting or inhibiting replication of microbes in host cells
- **Sepsis**: Regulating inflammatory response (i.e. inflammasome)
- **Lung cancer**: Promoting survival and proliferation of cancer cells, Tumor suppression
- **COPD**: Promoting apoptosis
- **IPF**: Regulating fibroblast activation and proliferation
- **PAH**: Regulating vascular cell proliferation