Chapter 14

The Role of Autophagy in Lung Disease

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Additional information is available at the end of the chapter

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Abstract

The autophagy degradation pathway is a cellular pathway that sequesters cargo from the cytosol to autophagosomes that are transferred to lysosomes for degradation or recycled as precursor metabolites. The autophagy pathway allows the removal of damaged organelles/proteins and is emerging as an important aspect of multiple human pulmonary diseases. The autophagy process is important in both the function of the immune system and the control of inflammation. Xenophagy (autophagy of bacteria) is an example of selective autophagy which could play a role in host defense mechanisms in pulmonary diseases such as sepsis. Autophagy pathways involving the degradation of cytosolic cargo could play different roles in disease pathogenesis and progression. In the case of certain lung diseases, mitophagy is elevated and the cilia shorten (ciliophagy), which contribute to lung dysfunction in the pathogenesis of chronic obstructive pulmonary disease. In other types of lung diseases such as pulmonary vascular disease, autophagy may provide a protective role to allow cell proliferation, repair and control of cell death. Disruption of autophagy in cystic fibrosis and idiopathic pulmonary fibrosis could promote pathogenesis of the disease. In lung cancer, autophagy is a 'double-edged sword' it blocks progression, but at the same time promotes tumor growth. In this chapter, we will review the different types of autophagy, the role of autophagy and its significance to human lung diseases. In addition, we will discuss the potential of targeting autophagy with therapeutics for lung disease management.

Keywords: autophagy, cancer, COPD, infection, CF

1. Introduction

Autophagy is an evolutionarily-conserved cellular mechanism that allows the turnover of organelles and proteins, through a lysosome-dependent degradation pathway in the cell. The

most common type of autophagy (also called macroautophagy) involves the sequestering of cytosolic molecules into double-membrane compartments called autophagosomes, which subsequently fuse to lysosomes where their contents are degraded and recycled into metabolic precursors (**Figure 1**). Autophagy is emerging as an important mechanism in the pathogenesis of several pulmonary diseases, including lung injury, sepsis, pulmonary vascular disease, idiopathic pulmonary fibrosis (IPF), cystic fibrosis and cancer. As autophagy can act as a modulator of pathogenesis, it is a therapeutic target. A number of studies indicate that autophagy may provide a protective function against disease and at the same time contribute to deleterious processes, usually when its activity is impeded or elevated. Autophagy is a known function in the clearance of subcellular material and the recycling of metabolites playing a role in cellular detoxification and adaptive or protective mechanisms. It is well accepted that autophagy plays a key role as a regulator of adaptive and innate immune responses, hence modulating autophagy could have a profound effect on disease pathogenesis. Xenophagy, which is the autophagic clearance of pathogens/bacteria, is one of the host's protective defense mechanisms against the pathogenesis of sepsis and inflammatory diseases. However, autophagy clearance of mitochondria may contribute to chronic obstructive pulmonary disease (COPD) through the activation of cell death pathways. In other types of pulmonary diseases, impairment of autophagy may aggravate and contribute to the pathogenesis process. In contrast, active autophagy in lung cancer could have many consequences including controlling carcinogenesis, modulating treatment efficacy and thus supporting tumor cell survival.

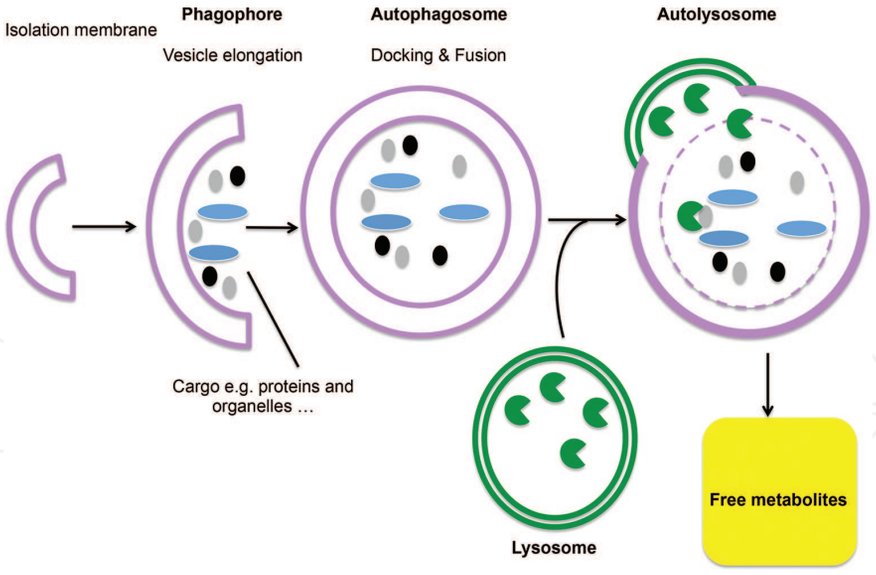

Figure 1. Schematic of the steps of autophagy. In the first step of autophagy the isolation membrane or phagophore is formed. In the next step of vesicle elongation, the proteins of the autophagy core machinery are believed to gather at the phagophore assembly site (PAS), and expand the phagophore into an autophagosome. In the next step, the autophagosome can engulf the cytosolic cargo including targeted cargo/organelles specifically. Following engulfment of cargo, the autophagosome can fuse with an endosome called an amphisome (not shown) or directly fuse with a lysosome to form an autophagolysosome. In the final step the cargo inside the autophagolysosome is degraded and free metabolites recycled.

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2. Autophagy regulation

The autophagy process is comprised of the rearrangement of membrane components derived from the endoplasmic reticulum (ER), the endosome/Golgi, plasma membrane and mitochondrial membranes [1–9]. Autophagy proceeds through several sequential steps (**Figure 1**), starting with the formation of the phagophore or the isolation membrane at the preautophagosome site (PAS, see **Figure 1**) [10]. Next, the autophagic membrane elongates forming a double-membrane autophagosome engulfing a part of the cytoplasm. The maturation of the autophagosome and its cargo results in their fusion with the lysosome to form a single-membrane compartment, the autolysosome. This process is assisted by many proteins such as class C Vps proteins, small GTPases (e.g., Rab5 and 7), UVRAG and lysosome-associated membrane proteins (e.g., LAMP2) [11–14]. The cargo can then be degraded through the action of lysosomal degradative enzymes such as acid hydrolases that are activated at low pH (pH 5). This is accomplished by a proton pump located in the lysosomal membrane pumping H+ ions from the cytosol into the lysosome [15]. The degraded products such as free amino acids, nucleotides and fatty acids are then released into the cytoplasm by lysosomal permeases and then recycled through anabolic pathways [10].

A large number of autophagy-related (Atg) proteins regulate autophagy through various steps of initiation and execution [16, 17]. Autophagy is regulated by upstream cellular signals such as glucose or amino acid starvation and is an adaptive response in these conditions [10]. Autophagy contributes to cellular homeostasis along with the unfolded protein response (UPR) and endoplasmic reticulum stress (ERS). All these cellular responses are linked through crosstalk and can be activated by external stimuli such as hypoxia [18]. The mammalian target of rapamycin complex 1 (mTORC1) and the energy sensing, 5′-adenosine monophosphate-regulated kinase (AMPK) are the main regulators initiated under starvation conditions. The mammalian target of rapamycin (mTOR) pathway suppresses autophagy under starvation conditions can be activated by growth factors through the Class I phosphatidylinositol-3-kinase (PI3K)/Akt-pathway [19]. As well as mTOR protein, mTORC1 is comprised of the regulatory-associated protein of mTOR (Raptor), the mammalian lethal with SEC13 protein 8 (mLST8), and the 40 kDa proline-rich Akt/PKB substrate (PRAS40) [17]. mTORC1 can be inhibited by rapamycin or starvation leading to autophagy activation via derepression of the substrate complex, the uncoordinated 51-like kinase-1 (ULK1) complex, which is comprised of ULK1, FIP200/RB1CC1, Atg13 and Atg101 [20–24]. Upregulation of AMPK is directly related to an increase in AMP levels which inturn downregulates mTORC1 by Raptor phosphorylation and in addition activates ULK1 [25, 26]. A recent study has shown that the trafficking of mAtg9 which is involved in vesicle delivery to the PAS is regulated by AMPK-dependent ULK1 phosphorylation [27].

The Beclin1 complex is comprised of Beclin1, class III PI3K (PI3KC3/Vps34), UVRAG or p150 and Atg14L [28, 29]. The connection of ULK1 and Beclin 1 complex regulation via Vps34 phosphorylation has been demonstrated [30, 31]. Also, Beclin 1 complexes are known to associate with a number of inhibitory or activating proteins such as Ambra 1 [28]. Autophagosome formation requires the activation of PI3KC3 in the Beclin 1 complex leading to the

formation of phosphatidylinositol-3-phosphate (PI3P). Various accessory protein factors are recruited by PI3P such as the double FYVE-containing protein-1 (DFCP1) and the WD-repeat protein in association with phosphoinositides (WIPI) proteins that are important in the assembly of autophagosomes [20, 32, 33]. In addition, the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins are also implicated recruitment of membrane and the assembly of autophagosomes [34, 35].

There are two ubiquitin-like conjugation systems: the LC3/ATG8 conjugation system and the ATG5–ATG12 conjugation system [36]. Autophagosome assembly requires ATG5–ATG12 conjugates along with ATG16L1. The ubiquitin-like protein microtubule-associated protein-1 light chain 3 (LC3B) mediates autophagosome formation [17]. The pro-LC3 form is cleaved by the endopeptidase, ATG4B generating LC3B-I form. In eukaryotes, LC3B-I (unconjugated form) conversion to its conjugated phosphatidylethanolamine (PE) form, and the association of LC3B-II with the membrane is an important stage of autophagosome biogenesis [36, 37]. LC3B-II association with the autophagosomal membrane is prolonged until the final stages of autophagosome-lysosome fusion. At the final stages, the LC3B-II associated with the outer membrane can be recycled by ATG4B while LC3B-II found on the inner membrane will be degraded by lysosome degradative enzymes [17].

3. Autophagy

3.1. Lung injury

Acute lung injury (ALI) is characterized by an uncontrolled acute inflammation and dysfunction of both the endothelial and epithelial barriers in the lung, and disproportionate transepithelial leukocyte migration, affecting the integrity of the alveolar-capillary membrane and the increase of proinflammatory cytokines [38–40]. In addition, ALI is linked to hyperoxia, sepsis, trauma, xenobiotic exposure and mechanical ventilation.

The annual incidence of ALI in the USA in 2005 was around 78.9 per 100,000 [41]. However, current ALI therapy is fairly basic involving simple medical or surgical treatment allowing an improvement in ventilation of the patient, while remedial ALI therapy exists [39, 42, 43]. The ALI pathogenesis can be rationalized by the injury to both the alveolar epithelium and vascular endothelium. The innate immune system cells are both the target of damage and effectors of injury in ALI/acute respiratory distress syndrome (ARDS). Damage to type I alveolar epithelial cells promotes pulmonary edema and the degradation of the epithelial layer revealing the basement membrane increasing the chances of bacteremia and sepsis. Damage of type II alveolar cells results in perturbed synthesis of surfactant and metabolism giving rise to elevated alveolar surface tension and alveolar collapse [38]. A diverse range of biomarkers such as intracellular adhesion molecule-1 (ICAM-1), the receptor for advanced glycation end-products (RAGE), von Willebrand factor (vWf) and surfactant D (SP-D) have been discovered in the endothelium and the epithelium; these play a role in coagulation cascades and inflammation and may be used to predict outcome in patients with ALI [44–47]. In addition, aberrations in the coagulation cascade as a result of plasminogen activator inhibitor-1 and protein C

also contribute to ALI [48]. Furthermore, the elevated levels of the inflammatory factors such as interleukins (IL-1, -6, -8) and tumor necrosis factor (TNF) are often associated with a response to cellular injury [49, 50].

Autophagy has been implicated in the pathogenesis of ALI [51] but could be due to long exposure to high levels of oxygen (hyperoxia) through mechanical ventilation treatment. Hyperoxia is sufficient to induce autophagy activation and LC3B knockdown effects cell survival [51]. Further studies have shown that in normal physiological conditions, the formation of a complex, p62/LC3B/truncated BH3-interacting domain death agonist (tBID) is necessary for cellular homeostasis [52]. In hyperoxia conditions, this complex dissociates and prevents the transport of tBID to the lysosome. The elevated levels of tBID result in the mitochondria releasing cytochrome c and caspase-dependent cell death [52]. The outcome of hyperoxia can lead to mitochondrial dysfunction in the lungs [53, 54]. Thus, mitophagy in hyperoxia has been implicated to play a role in ALI pathogenesis [55].

3.2. Infectious lung disease

Phagocytosis can act against infection in two ways: by ingesting bacteria (by macrophages and neutrophils) or by processing infectious agents for antigen presentation by dendritic cells. Similarly, autophagy is emerging as an essential process in innate and adaptive immune functions [56]. Autophagy plays an important role in protection of the host from various microbes such as viruses, bacteria and parasites [56–58]. The functions of antibacterial and antipathogenic autophagy are well characterized [59, 60]. It is known that phagocytosis of nonpathogenic mycobacteria by macrophages stimulates autophagy and apoptosis resulting in the removal of the pathogen. Conversely, the phagocytosis of pathogenic mycobacteria can perturb the autophagy pathway [61].

Tuberculosis is the result of a pathogen infection called *Mycobacterium tuberculosis* which is a serious infection affecting the lungs and is a burden worldwide [62]. The mycobacteria are resistant, remaining and replicating inside the immature phagosomes during tuberculosis infection. *M. tuberculosis* utilizes a protective survival strategy which allows them to interfere with a fusion event at the phagosomal compartments comprising *M. tuberculosis* and lysosomes [63]. Furthermore, the *M. tuberculosis* phagocytosis stimulates necrotic cell death allowing mycobacteria release to uninfected cells as opposed to stimulating macrophage apoptosis. Thus, this leads to a reduction in mycobacterial antigen presentation and promotion of *M. tuberculosis* infection [64]. The intracellular survival and replication rate of *M. tuberculosis* can be reduced through stimulation of autophagy [59, 60, 64–66]. Recent studies have shown that several compounds that stimulate autophagy through mTORC1 inhibition to alleviate *M. tuberculosis* infection [67, 68]. At the same time, autophagy inhibition promotes *M. tuberculosis* infection [60].

Autophagy can be important for antivirulence elements against *M. tuberculosis* generated substrate degradation [69, 70]. The host defense system against *M. tuberculosis* is protected by the production of interferon-gamma (IFN-γ) which stimulates macrophages, induces the autophagy process and thus protects the host against infection [60, 69]. In fact, stimulating with IFN-γ leads to bacteria degradation by p62-dependent selective autophagy [69]. Autoph-

agy induced by IFN-γ requires p47 guanosine triphosphatase IRGM-1 [60, 71, 72] and increased sensitivity to *M. tuberculosis* infection is associated with single nucleotide polymorphisms in the IRGM-1 gene [73].

M. tuberculosis processing has been recently linked to selective autophagy [74]. It is known that the bacterial early secretory antigenic target (ESAT-6) system 1 (ESX-1) secretion system facilitates phagosomal permeabilisation allowing the ubiquitin-mediated autophagy entry to phagosomal *M. tuberculosis*. The extracellular bacterial DNA is recognized and tagged with ubiquitin by the stimulator of interferon gene (STING)-dependent cytosolic pathway. Then p62 and NDP52, the autophagy cargo adaptors, recognize ubiquitinated *M. tuberculosis* and target them to autophagosomes.

Autophagy is also described as a defense mechanism against other pathogens affecting the respiratory system. The growth of *Legionella pneumophila* which causes Legionnaire's disease is promoted in the absence of Atg9 suggesting that autophagy contributes to defense against this pathogen [75]. Influenza-A virus infection induces autophagy and autophagosome formation is necessary for virus replication [76], but it is believed that the influenza-A proteins such as M2 protein, can block autophagosome formation and subsequent fusion with the lysosome [77]. Recent studies on influenza infection have shown that autophagy is a requisite for maintaining memory B cells that are necessary in secondary antibody responses. Mice carrying a B cell-specific knockout of Atg7 showed perturbed secondary antibody responses and thus were more sensitive to influenza infection [78]. Therefore, future therapeutic developments should challenge the modulation of the autophagy pathway to eliminate infection and encourage adaptive immunity against infectious agents.

3.3. Emphysema and chronic obstructive pulmonary disease

Emphysema is characterized by the deterioration of the lung parenchyma as a result of an abnormal inflammatory response. In addition, there is degradation of the pulmonary matrix leading to an increase in the lung space and reduced respiratory function [79]. It is commonly observed that mice exposed to cigarette smoke give rise to an increase in lung space and can be used as a model of emphysema. Mice exposed to cigarette smoke over 3 months demonstrate raised autophagosomal numbers and elevated levels of LC3B-II in the resected lung tissue [80]. Interestingly, in the LC3B knockout mouse model demonstrated insensitivity to cigarette smoke leading to no change in the lung space. Depletion of the early growth response-1 (Egr-1) known to regulate LC3B transcription resulted in an increase in lung space with no changes induced with cigarette smoke. Conversely, knockout of caveolin-1 in mice led to sensitivity to cigarette smoke [80, 81]. Thus, LC3B is considered to be a crucial regulator of apoptosis and autophagic signaling.

Chronic obstructive pulmonary disease (COPD) is emerging as a global burden to society [82]. COPD is clinically defined into different types of lung disease, including emphysema and bronchitis leading to obstruction of the airway with mucus [83]. The main risk factor for COPD is cigarette smoke [84]. The mechanisms of COPD pathogenesis are still unknown. However, some theories indicate that abnormal cellular and inflammatory responses to cigarette smoke within the lung, the vessels and the airspace may be involved [84, 85]. The role of autophagy

in COPD pathogenesis could be demonstrated by an excessive increase in autophagy or by mitophagy (selective degradation of mitochondria by autophagy) leading to cell death [80, 81, 86–88]. These studies indicate that the levels of the autophagy markers (such as LC3-II) and the mitophagy markers (such as PINK1) are elevated in COPD patient's lung tissue. Thus, an increase in autophagy or a defect in the autophagy flux could contribute to COPD pathogenesis [80, 87, 89]. In fact, a number of studies have shown that cigarette smoke can induce abnormal autophagy and mitophagy leading to bronchial cell death through apoptosis or necroptosis, respectively [81, 87]. Furthermore, there was an increase in histone deacetylase-6 levels as a result of oxidative stress, inducing hypomethylation leading to autophagy and cilia shortening which could contribute to mucociliary dysfunction [86]. Previous studies have shown that a block in autophagy promotes cilia growth [90]. Cigarette smoke has been shown to activate autophagy leading to elevated cell senescence along with a build-up of both p62 and ubiquitinated proteins [91]. As a result of autophagy blockade, cells display a 'senescence-associated secretory phenotype' secreting interleukin 8 (CXCL8) [91]. Autophagy was rescued with Torin 1, an mTOR inhibitor [91]. The genetic knockout Atg5−/− mouse model showed that p62 levels were elevated in the lung epithelial cells along with a shortening of cilia [92].

A recent study analyzing autophagy in macrophages in the alveoli from smokers' lung showed induction of LC3-II and the presence of autophagosomes [93]. The levels of autophagosomes in macrophages were elevated and autophagy activity was perturbed [93]. Altogether, these studies implicate that autophagy is an important cellular process in COPD pathogenesis. Future studies will need to address whether cigarette smoke promotes or blocks the autophagy flux in lung parenchyma, as well as the links between autophagy, apoptotic cell death and development of emphysema.

3.4. Pulmonary vascular disease

Hypoxia is an important contributing factor to pulmonary cardiovascular diseases such as pulmonary hypertension (PH), ischemia-reperfusion injury and atherosclerosis [94]. The hypoxia-inducible factors (HIFs), heterodimers of transcription family Per-Arnt-Sim/basic helix-loop-helix, regulate hypoxia. The heterodimer of HIF-1 is composed of two subunits, HIF-1β and HIF-1α, the O2-sensitive subunit. Under normoxia conditions, HIF-1α is degraded by the proteasome aided by HIF prolyl hydroxylase and the von Hippel-Lindau E3 ubiquitin ligase [94].

It is widely reported that the autophagy process can be regulated by hypoxia in cells and tissues. This process could be a crucial factor in cell injury induced by hypoxia in mitochondrial turnover [95–98]. Under *in vitro* conditions, autophagy activation requires Beclin 1 and an element that can be induced, encompassing HIF-1α stabilization and elevated ROS production [67, 96]. In addition, hypoxia-inducible autophagy is regulated by BNIP3 (Bcl-2/adenovirus E1B 19 kDa-interacting protein-3), a Bcl-2 family member [96].

The accumulation of abnormal, unfolded proteins during hypoxia or nutrient deprivation (endoplasmic reticulum stress, ERS) is the stimulus for the unfolded protein response. In its adaptive phase, this vital defense mechanism initially recruits autophagy to assist ER-associated degradation in the breakdown of abnormal proteins, while blocking cell death

pathways. This prosurvival role for autophagy is partly responsible for resistance of hypoxic cells to therapies, such as chemotherapy [18]. Autophagy activation in tumor cells through hypoxia requires BNIP3 [97, 98]. In this case, autophagy could provide a mechanism of cell survival in hypoxia conditions via selective mitophagy [96]. It still remains unclear how BNIP3 acts during hypoxia either through cell survival or the cell death pathway.

Hypoxia conditions trigger autophagy in pulmonary vascular primary cells. In particular, hypoxia experiments performed *in vitro* show that LC3B-II accumulates and Beclin 1 expression increases both in pulmonary vascular endothelial cells and muscle cells. In addition, GFP-LC3 puncta are formed which is a common feature of autophagosome formation in cells transfected with GFP-LC3 [95]. In the same study, LC3-II and autophagosome formation were elevated in the presence of the autophagosomal inhibitor, bafilomycin A1, indicating elevated autophagic activity [95].

3.5. Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is characterized by the build-up of extracellular matrix proteins deposited in the interstitial tissue and basement membrane of damaged epithelium and an increase in active mesenchymal cells such as myofibroblasts [99]. The lung interstitium is primarily composed of fibroblasts, which play a role in maintaining the extracellular matrix and in wound healing. Altered autophagic function is implicated in the pathogenesis of IPF. Elevated cellular senescence and decreased autophagy activity as a result of decreased LC3B protein expression have been observed in the lung tissues from patients with IPF and in lung fibroblasts treated with TGF-β [91, 100]. TGF-β1 is known as an inhibitor of autophagy in human lung fibroblasts. In fibroblasts, genetic ablation of the autophagy proteins, LC3B or Beclin1, elevates the expression of fibronectin and α-smooth muscle actin (a myofibroblast marker) induced by TGF-β1 [100]. The mTORC1 inhibitor, rapamycin, was shown to protect against lung fibrosis when mice were treated [100]. Thus, reduced autophagy in IPF patients could increase the efficacy of TGF-β1 leading to a build-up of the extracellular matrix and conversion to a myofibroblast phenotype. Perturbation of lung IL-17A has been demonstrated to be protective against fibrosis, through autophagy recovery, in a mouse bleomycin model of pulmonary fibrosis [101]. Recent studies have shown that the multiple tyrosine kinase inhibitor nintedanib downregulated extracellular matrix production and promoted autophagy in IPF fibroblasts while inhibiting the TGF-β signaling pathway [102]. However, further investigations are required to better understand the role of autophagy and the molecular mechanism of fibrogenesis.

3.6. Cystic fibrosis

CF is a fatal genetic lung disease caused by a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR); it is characterized by the accumulation of mucus in the airways. This accumulation can result in damage to the lungs that are linked to recurrent secondary infections. The CFTR mutation of a deletion of phenylalanine at position 508 (CFTRF508del) is the most common found in humans [103]. Recent studies are demonstrating defective autophagy with the CFTR mutation in airway epithelial cells from CF

patients with the CFTR mutation. This is visualized by a reduction in autophagosome number along with the presence of LC3 puncta and p62 accumulation in starvation conditions [104]. Cells expressing CFTR F508del have been shown to be defective in autophagy and to accumulate polyubiquitinated proteins and aggresome-like structures [105]. Inflammatory response is enhanced in CF with defective autophagy [106]. In addition, defective CFTR can upregulate reactive oxygen species production and tissue transglutaminase [104]. A block in autophagy leads to cross-linking and inactivation of Beclin1 with PI3KC3 sequestering and p62 accumulation [104]. Protein trafficking of CFTR F508del to the cell surface could be restored with the overexpression of Beclin1 or the depletion of p62 [104]. Targeting p62 genetically rescued the functional expression of CFTR and improved the efficacy of CFTR channel activators [107]. Genetic targeting of p62 was also recently shown to improve the therapeutic effect of CFTR channel activators [107].

Xenophagy, the clearance of bacteria by autophagy, could play a role in defending against infections linked to CF. In a mouse CF model, rapamycin has been shown to alleviate *Burkholderia cenocepacia* infection and reduce lung inflammation [108]. *In vivo* studies have shown that pharmacological intervention of autophagy could enhance the clearance of *Pseudomonas aeruginosa* bacteria from the lung [109]. Therefore, dysfunctional autophagy leading to a compromise as a result secondary infection could promote CF pathogenesis. Hence, future CF therapy should include the rescue of autophagy [110].

3.7. Cancer

The autophagy pathway is a complex biological process that is believed to influence the induction, development and cancer therapy. Autophagy is the main cellular protection system with an anticarcinogenic effect through preserving mitochondria, recycling of precursors of metabolism, removal of cell debris/products and the control of inflammation that could promote gene instability [111]. Surprisingly, autophagy could provide a mechanism that contributes to tumor cell survival and cancer progression as a result of chemotherapy resistance. At the same time, autophagy could provide a means to treat the cancer through chemotherapy promoting autophagy-mediated cell death [111].

Early studies demonstrated that the monoallelic loss of Beclin1 from chromosome 17q21 occurs in many cancers such as prostate, breast and ovarian [112–114]. It is reported from *in vitro* studies that perturbing autophagy can promote cancer growth, and in addition heterozygous Beclin1 mice develop lung, liver and lymphomas [115, 116]. In addition, the aberrant expression of Beclin1 in the tumor is associated with poor outcome and aggressive disease [57, 58]. Currently, the role of autophagy in the lung and its effect on therapy is an unexplored area of research. Studies have shown that Beclin1 expression is reduced in NSCLC compared to healthy tissue and this reduction is further pronounced in higher stage, poorly differentiated tumors [117, 118]. Autophagy induction with mTOR inhibitors is linked to radiosensitization in NSCLC [119]. In addition, hydroxychloroquine, an autophagy inhibitor, has been tested in the clinic for treatment of NSCLC on the basis that inhibition of autophagy, which aids cell-survival, helps prevent resistance to chemotherapy [113]. Depletion of the autophagy protein, ATG5, both perturbs the progression of KRas (G12D) lung cancer and stimulates tumor

survival in a mouse model. On the other hand, ATG5 depletion promotes the initiation of KRas (G12D) tumors [120]. These studies indicate the 'double-edged sword of autophagy' in cancer where autophagy prevents progression and promotes tumor growth.

4. Conclusion and future perspectives

It is emerging that autophagy can promote and perturb pathogenesis in human disease (see **Figure 2**). These effects are well illustrated in cancer, where the 'double-edged sword' of

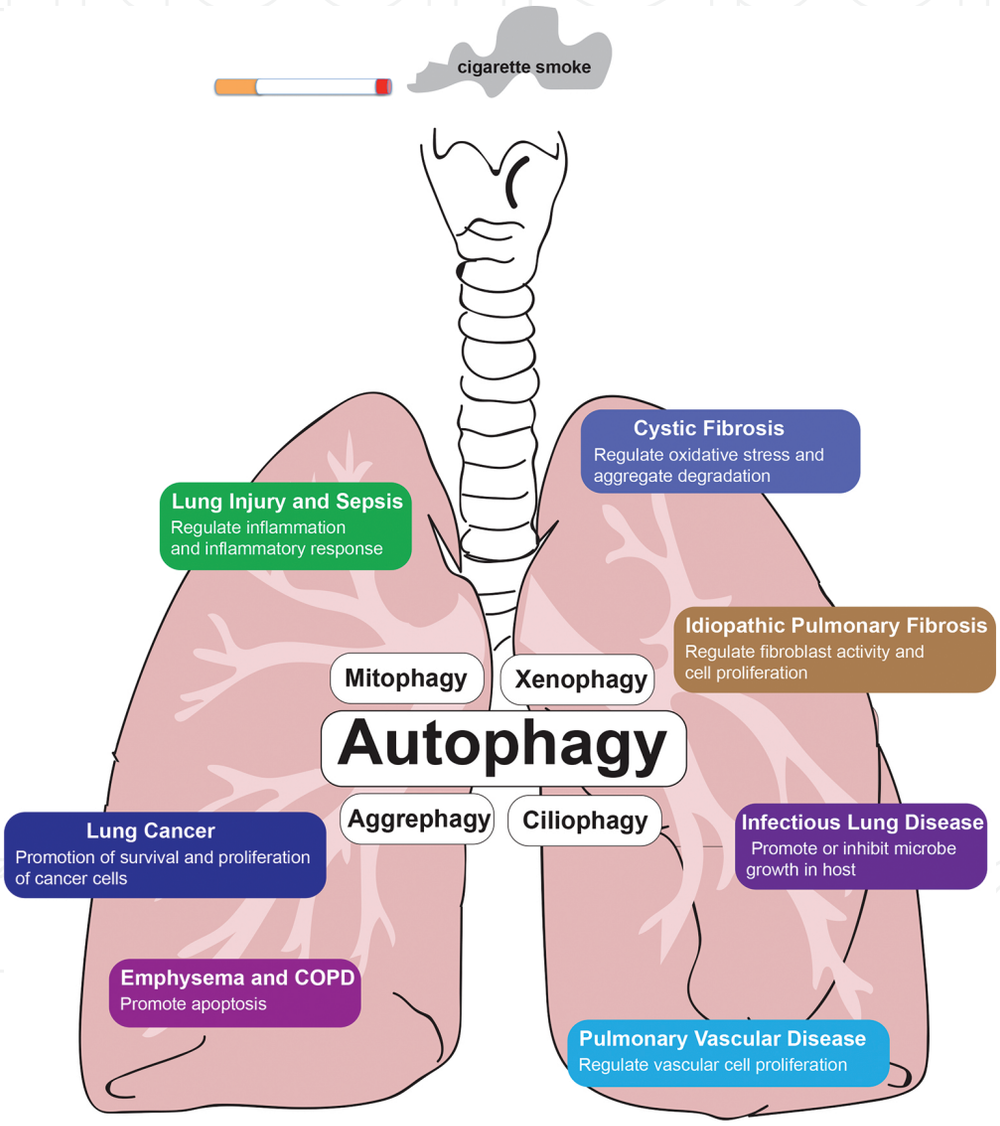

Figure 2. The effects of autophagy in human lung diseases. The role of different pathways of autophagy to remove bacteria (xenophagy), mitochondria (mitophagy), cilia (ciliophagy) and protein aggregates (aggrephagy). The autophagy process plays a role in the pathogenesis of many human lung diseases as shown in the schematic. In lung cancer, autophagy could perturb tumorigenesis, but at the same time promote cell proliferation and thus tumoral survival. In pulmonary infections, autophagy could reduce bacterial expansion and block survival of bacteria such as *M. tuberculosis*. The pathogenesis of human lung disease is promoted with cigarette smoke exposure which induces oxidative stress and triggers cilia protein damage.

autophagy provides protection in the early stages of disease, while in the later stages promotes tumor growth or resistance to therapy. In some lung diseases, such as sepsis, autophagy could provide protection against bacterial infection and allow an inflammatory response. In the case of exposure to cigarette smoke, this aggravates and promotes disease progression for many human lung diseases. Current therapeutic strategies are still in development, and a few molecules that regulate autophagy have already been tested in the clinic. For example, the autophagy inhibitors chloroquine and hydroxychloroquine and autophagy inducer, mTOR inhibitor rapamycin, have been analyzed in the clinic. Recent development of compounds that modulate autophagy has been the Tat-Beclin 1 peptide, an inhibitor of histone deacetylases [121], vitamin D and AMPK activators [58, 113, 122]. Due to the complexity of autophagy, further research is required to fully understand how modulating autophagy could be used as a therapeutic strategy in disease management. In addition, we will need to define the various pathways of selective autophagy and their relevance to disease pathogenesis. This will allow the design of novel compounds to be used in therapeutic strategies that could specifically target certain lung diseases.

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