# Pathophysiologic Role of Autophagy in Human Airways

**Valentina Sica and Valentina Izzo**

**Abstract** Lung diseases are among the most common and widespread disorders worldwide. They refer to many different pathological conditions affecting the pulmonary system in acute or chronic forms, such as asthma, chronic obstructive pulmonary disease, infections, cystic fibrosis, lung cancer and many other breath complications. Environmental, epigenetic and genetic co-factors are responsible for these pathologies that can lead to respiratory failure, and, even, ultimately death. Increasing evidences have highlighted the implication of the autophagic pathways in the pathogenesis of lung diseases and, in some cases, the deregulated molecular mechanisms underlying autophagy may be considered as potential new therapeutic targets. This chapter summarizes recent advances in understanding the pathophysiological functions of autophagy and its possible roles in the causation and/or prevention of human lung diseases.

## Abbreviations

| | |
|---|---|
| AAT | Alpha-1-antitrypsin |
| AATD | Alpha-1-antitrypsin deficiency |
| ALI | Acute lung injury |

V. Sica
Ecole doctorale de Cancerologie, University of Paris SUD, Paris, France

"Apoptosis, Cancer & Immunity", Team 11, INSERM U1138 Cordeliers Research Center, 15 rue Ecole de Médecine, 75006 Paris, France

Cell Biology and Metabolomics Platform, Gustave Roussy Comprehensive Cancer Center, Pavillon de Recherche 1, 114 rue Édouard-Vaillant, 94805 Villejuif, France

V. Izzo (✉)
"Apoptosis, Cancer & Immunity", Team 11, INSERM U1138 Cordeliers Research Center, 15 rue Ecole de Médecine, 75006 Paris, France

Cell Biology and Metabolomics Platform, Gustave Roussy Comprehensive Cancer Center, Pavillon de Recherche 1, 114 rue Édouard-Vaillant, 94805 Villejuif, France
e-mail: valentina.izzo@upmc.fr



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| | |
|---|---|
| ALT-E | Alternaria-associated asthma |
| ARDS | Acute respiratory distress syndrome |
| Atg | Autophagy-related |
| ATP | Adenosine triphosphate |
| Bcl-2 | B-cell lymphoma 2 |
| BMP | Bone morphogenetic protein |
| BMPR2 | BMP receptor type-II |
| BRAF | B-Raf proto-oncogene |
| CAV-1 | Caveolin-1 |
| CD274 | Cluster of differentiation 274 (known as Programmed death-ligand 1, PD-L1 or B7 homolog 1, B7-H1) |
| CF | Cystic Fibrosis |
| CFTR | Cystic Fibrosis Transmembrane Conductance Regulator |
| COPD | Chronic obstructive pulmonary disease |
| CRC | Murine colorectal carcinoma |
| CS | Cigarette smoke |
| ECM | Extracellular matrix |
| EGFR | Epidermal growth factor receptor |
| Egr-1 | Early growth response protein 1 |
| EMT | Epithelial-to-mesenchymal transition |
| ER | Endoplasmic Reticulum |
| F508del-CFTR | Deletion of phenylalanine in position 508 of the CFTR |
| FEV1 | Forced expiratory volume in 1 second |
| FF | Fibroblastic foci |
| FMD | Myofibroblast differentiation |
| FoxO3 | Forkhead box O3 |
| FOXP3 | Forkhead box P3 |
| $H_2O_2$ | Hydrogen peroxide |
| HDAC6 | Histone deacetylase 6 |
| HH | Hedgehog |
| HO-1 | Heme oxygenase-1 |
| IFN | Interferon |
| IFT20 | Intraflagellar transport protein 20 homolog |
| IL | Interleukin |
| ILD | Interstitial lung disease |
| IPF | Idiopathic pulmonary fibrosis |
| KRAS | Kirsten rat sarcoma viral oncogene homolog |
| LC3 (MAP1LC3) | Microtubule-associated protein 1 light chain 3* |
| LPS | Lipopolysaccharide |
| MCC | Mucociliary clearance |
| MMP | Matrix metalloproteinases |
| mTOR | Mammalian target of rapamycin |
| MUC5AC | Mucin 5AC |
| MyD88 | Myeloid differentiation primary response gene 88 |
| NK | Natural killer |

| | |
|---|---|
| NO | Nitric oxide |
| NSCLC | Human non-small cell lung carcinoma |
| OFD1 | Oral facial digital syndrome |
| p62/SQSTM1 | Sequestosome 1 |
| PAH | Pulmonary arterial hypertension |
| PARK2 | Parkin RBR E3 ubiquitin protein ligase |
| PASMCs | Pulmonary artery smooth muscle cells |
| PH | Pulmonary hypertension |
| PI3K | Class III-phosphoinositide 3-kinase |
| PINK | PTEN-induced putative kinase |
| PTEN | Phosphatase and tensin homolog |
| ROS | Reactive oxygen species |
| Rtp801 | Known as Redd1 (regulated in development and DNA damage responses 1) |
| SIRT6 | Sirtuin 6 |
| SNPs | Single Nucleotide Polymorphisms |
| STK11 (LKB1) | Serine/threonine kinase 11 |
| TFEB | Transcription factor EB |
| TG2 | Transglutaminase type 2 |
| TGF-β1 | Transforming growth factor-β1 |
| Th | T helper |
| TLR4 | Toll-like receptor 4 |
| TSC | Tuberous sclerosis complex |
| WHO | World Health Organization |
| α-SMA | Smooth muscle-α actin |

## 1 Introduction

Lung diseases are some of the most common medical conditions in the world. The lung has the principal aim to mediate gas exchange [60]. For this reason, the lung can be subjected to several insults, belonging to the environment (inspiration of foreign matter, particles, smoke), reactive oxygen species (ROS) production, biological origins (e.g., viruses, bacteria), changes in $O_2$ tension, and mechanical stresses (e.g., mechanical ventilation). It is possible to discriminate between diseases affecting: (I) the airways (asthma, chronic obstructive pulmonary disease, chronic bronchitis, emphysema, acute bronchitis and cystic fibrosis); (II) the interstitium (sarcoidosis, idiopathic pulmonary fibrosis, autoimmune diseases, pneumonias and pulmonary edemas); (III) the blood vessels (pulmonary embolism and hypertension); the pleura (pleural effusion, pneumothorax and mesothelioma); (IV) the chest wall (obesity hypoventilation syndrome and neuromuscular disorders). The development of lung diseases can be associated to both acute and chronic exposure to such insults. However, in most conditions, a favouring genetic is necessary [60]. Yet, the lung has

various inducible defence mechanisms to protect itself. First, constitutive and inducible stress protein and antioxidant defences; second, innate immune responses; third, pro- and anti-apoptotic mechanisms [84, 85, 103]. Several studies have recently pinpointed the emerging role of macroautophagy (more often and hereby referred to as autophagy) in lung homeostasis and diseases. Autophagy is a catabolic process that involves the sequential sequestration of cytoplasmic material within double-membraned vesicles (autophagosomes), the fusion of autophagosomes with lysosomes, and the degradation of autophagosomal cargoes (as well as of structural autophagosomal components) by lysosomal hydrolases [26]. Autophagy is mediated by a genetically encoded, evolutionary conserved machinery that is connected to most, if not all, major biochemical processes of the cell, including core metabolic circuitries as well as signal transduction pathways initiated by plasma membrane receptors [18]. Basically, autophagy responds to three major organismal needs: (1) it preserves cellular homeostasis in physiological conditions; (2) it plays a key role in cellular adaptation to stressful stimuli; and (3) it participates in the communication of states of the danger to the whole organism [21]. Indeed, autophagy continuously operates to mediate the disposal of potentially dangerous structures that may otherwise accumulate in the cytoplasm as a consequence of normal cellular activities, like old (and damaged) organelles or protein aggregates [64]. Moreover, the autophagic flux is highly responsive to situations in which intracellular or extracellular homeostasis is perturbed, which generally involves either an increased offer of autophagic substrates (as it occurs in the course of viral infection) or an increased need for autophagic functions or products (as it occurs in response to nutrient deprivation) [90]. In both these settings, proficient autophagic responses are required for the optimal adaptation of cells to stress, as demonstrated in experiments involving pharmacological inhibitors of autophagy or the depletion of essential components of the autophagic machinery [46]. Finally, autophagy is required for cells experiencing so-called "oncogenic stress" (i.e., the boost of cellular functions driven by activating mutations in one oncogene or loss-of-function mutation in one tumor suppressor gene) to become senescent (a cell-intrinsic oncosuppressive mechanism) while secreting immunostimulatory cytokines and expressing on their surface ligands for activatory natural killer (NK)-cell receptors (hence triggering a cell-extrinsic mechanism of tumor suppression) [55]. Along similar lines, cancer cells succumbing to a peculiar form of apoptosis known as "immunogenic cell death" are able to recruit antigen-presenting cells and hence trigger an adaptive immune response only if they secrete ATP as they die, a process that requires proficient autophagic responses [42, 45]. It should be noted that autophagy has also been causally implicated in some instances of cell death, especially in lower organisms like *Drosophila melanogaster* [13, 17]. However, in mammals autophagy mainly mediates robust cytoprotective functions, and – when cellular homeostasis is irremediably compromised – contributes to the maintenance of organismal homeostasis by playing a role in danger signalling. In line with this notion, defects in the autophagic machinery have been associated with a wide panel of human pathologies, including (but not limited to) malignant diseases, neurodegenerative disorders, as well as cardiovascular, renal and pulmonary conditions [86]. An accurate description of the autophagy pathway and its role in

immunity and inflammation has been provided in several previous chapters of this book; therefore, here we will focus on the impact of autophagic in the etiology and treatment of human pulmonary diseases.

## 2 Acute Lung Injury

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) describe clinical syndromes of acute respiratory failure with substantial morbidity and mortality. ALI is characterised by acute inflammation that causes disruption of the lung endothelial and epithelial barriers. The ALI cellular features include loss of alveolar–capillary membrane integrity, excessive transepithelial neutrophil migration, and release of pro-inflammatory, cytotoxic mediators. The treatment of ALI is predominantly based on ventilatory strategies [35]. However, prolonged exposure to high oxygen therapy (hyperoxia) can result in lung injury [7]. Few studies are present in the literature concerning the role of autophagy in ALI, even so these works support the hypothesis that activation of autophagy has a protective role in this disease. It has been demonstrated that prolonged hyperoxia, which causes characteristic lung injury in mice, induced the increase of LC3II expression. Moreover, in pulmonary epithelial cells, the genetic depletion of LC3 sentitizes the cells to hyperoxia-induced cell death suggesting that LC3 activation confers cytoprotection in oxygen-dependent cytotoxicity [93]. Besides, the involvement of mitophagy has also been identified. The ability to resist hyperoxia is proportional to PTEN-induced putative kinase 1 (PINK1) expression. In fact, the *Pink1*$^{-/-}$ mice were more susceptible to hyperoxia when compared to wild-type mice. Furthermore, genetic deletion of PINK1 or PINK1 silencing in the lung endothelium cells increased susceptibility to hyperoxia *via* alterations in autophagy/mitophagy, proteasome activation, apoptosis and oxidant generation [108].

## 3 Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease that causes breathing difficulty, cough, sputum production and dyspnoea. Emphysema and chronic bronchitis can contribute to COPD development. Emphysema is a condition resulting from a severe damage of air sacs (the alveoli). Chronic bronchitis is due to inflammation of the lining of the bronchial tubes. The lung damage that leads to COPD is caused by long-term exposure to irritating gases or particulate matter, most often from cigarette smoke (CS), air pollution or workplace exposure to dust, smoke or fumes. However, a genetic susceptibility to the disease should be considered as an important cofactor. Patients with COPD present increased risk of developing other pathologies, such as heart disease or lung cancer [53]. Multiple molecular mechanisms, not fully understood, participate to the

COPD evolution and, among others, the involvement of the autophagic pathway has been pointed out [3, 86]. In lung tissue from COPD patients, an increase of autophagic vacuoles as well as several autophagy markers (LC3, ATG4, ATG5/12, ATG7) expression has been detected [8]. These evidences are perhaps a result of defective autophagic flux. To corroborate this hypothesis, an increased accumulation of p62 and ubiquitinated proteins and a decreased expression levels of sirtuin 6 (SIRT6) have been evaluated in lung homogenates from COPD patients [92]. Kuwano and colleagues hypothesize that the insufficient autophagic clearance is involved in the accelerated cell senescence observed in COPD [16, 92]. The CS induces mitochondrial damage, accompanied by increased ROS production *in vitro*. The CS-induced mitophagy was inhibited by PINK1 and PARK2 knockdown, resulting in enhanced mitochondrial ROS production. Moreover, a decreased expression of PARK2 in COPD lungs compared with non-COPD lungs has been detected, suggesting that insufficient mitophagy is a part of the pathogenic sequence and cellular senescence of COPD [32]. In addition, a defective xenophagy has been observed in alveolar macrophages of smokers, suggesting that the deregulation of this selective process may contribute to recurrent infections [65]. In contrast, other findings indicate that autophagy has an opposite role in COPD favouring the pathological environment. It has been shown that Rtp801 (also known as Redd1) expression is increased in human emphysematous lungs and in lungs of mice exposed to CS, whereas Rtp801 knockout mice were protected against acute CS-induced lung injury. Rtp801 inhibits mammalian target of rapamycin (mTOR), by stabilizing the TSC1-TSC2 inhibitory complex. The inhibition of mTOR is linked to autophagy induction, but Rtp801 expression enhances oxidative stress-dependent cell death, amplifying the development of CS-induced lung injury [105]. Furthermore, the higher expression of autophagy proteins has been linked to lung epithelial cell death, airway dysfunction and emphysema in response to CS. Genetic depletion of LC3B *in vivo* (*Map1lc3B*$^{-/-}$ mice) suppressed cell death and emphysematous airspace enlargement during chronic CS exposure compared to the wild type mice [9]. More recently, the same group demonstrated that mitophagy regulates necroptosis, which contributes to the COPD pathogenesis. Mice deficient for *Pink1* were protected against mitochondrial dysfunction, airspace enlargement and mucociliary clearance (MCC) disruption during CS exposure [63]. Interestingly, they identified the contribution of a novel selective autophagy-dependent pathway that regulates cilia length, "ciliophagy", in the COPD pathophysiological evolution. Exposure to CS reduced cilia length and autophagy-impaired (*Beclin 1*$^{+/-}$ or *Map1lc3B*$^{-/-}$) mice resisted to the CS-induced cilia shortening *via* a mechanism involving histone deacetylase 6 (HDAC6) [48]. Accordingly, it has been shown that autophagy negatively regulate ciliogenesis by the degradation of the essential ciliary protein IFT20 [70]. Conversely, Hedgehog (HH) signalling from primary cilia promotes autophagy [70] and autophagy promotes ciliogenesis by degrading OFD1 (oral facial digital syndrome) at centriolar satellites [95]. Further studies are necessary to clarify the dual relationship between these processes [101]. In conclusion, these studies illustrate that the contribution of autophagy in COPD pathophysiology is complex and show a context-specific role depending on the cell type and tissue as well as on the different stimuli involved.

## 4 Interstitial Lung Disease (ILD)

Interstitial lung disease (ILD) is a general category that includes all lung diseases affecting the interstitium, the tissue and space that extends throughout both lungs. Among them the most common are Sarcoidosis and Idiopathic pulmonary fibrosis (IPF). Sarcoidosis is a systemic inflammatory disease caused by persistent reaction toward a stimulus (virus or antigens) that continues even when it is physiologically cleared from the body. Lung interstitium fibrosis is the first symptom in patients with Sarcoidosis. Conversely, IPF is characterized by specific fibrosis at interstitial level due to the increased extracellular matrix (ECM) protein deposition and hyper activation of myofibroblasts [10].

Recently, reduced LC3II expression and p62 accumulation has been found in lung tissue from IPF patients [72]. The reduced expression of the transcription factor FoxO3a in IPF fibroblasts could be the cause for the reduction in the levels of LC3 protein as the expression of this latter is positively stimulated by FoxO3a [30].

Furthermore, in fibroblast of IPF patients, decreased expression in Beclin-1 protein and increased expression of the anti-apoptotic protein Bcl-2 have been found, confirming a defect in the autophagy pathway at different level [81]. Moreover, fibroblastic foci (FF), that are the starting point for fibrogenesis, are enriched in ubiquitinated proteins and p62, confirming the insufficient autophagy at the basis of IPF pathogenesis [3].

Autophagy inhibition is able to induce acceleration of epithelial cell senescence and fibroblast to myofibroblast differentiation (FMD), which have a critical role in IPF development [3]. Transforming growth factor-β1 (TGF-β1) is one of the essential mediators of fibrosis since it stimulates fibroblasts to produce fibronectin and the smooth muscle-α actin (α-SMA), which is a myofibroblast marker. Autophagy has been associated to fibrosis through TGF-β1. In fact, genetic deletion of LC3 or Beclin 1 increases TGF-β1 activity as well as *in vivo* treatment with Rapamycin can protect from fibrosis [72]. TGF-β1 expression seems to be dependent on IL-17A, a proinflammatory cytokine involved in chronic inflammation and autoimmune disease. Blocking IL-17A might reduce the progression of fibrosis promoting the autophagic degradation of collagen [61].

Recently, lacking of matrix metalloproteinases-19 (MMP-19) has been associated with exacerbated fibrosis in the hyperplastic alveolar epithelium of IPF lungs [106]. Additionally, MMP-19-deficient mice exhibit diminished Atg4c protein expression, demonstrating a direct correlation between these two pathways [33]. Similar evidences from an independent group corroborate the role of autophagy in promoting FMD. In fact, Atg4b-deficient mice exhibited reduction in autophagic activity in lungs, collagen accumulation and increased protein levels of the myofibroblast biomarker α-SMA [6].

Pharmacological treatment with the alkaloid Barberine has been proposed for IPF monitoring because of its capacity to inhibit the activation of mTOR and to increase the expression of LC3 and Beclin 1 in an bleomycin *in vivo* model of airway-fibrosis [11]. Furthermore, the multiple tyrosine kinase inhibitor Nintedanib

has recently been approved for the treatment of IPS for its anti-fibrotic effect. It has been shown that Nintedanib is able to reduce the expression of ECM proteins, fibronectin and collagen as well as to induce a Beclin 1 dependent, ATG7 independent autophagy [76].

## 5 Asthma

Asthma is a chronic respiratory disease affecting 300 million people worldwide. Asthma manifests through several symptoms including wheezing, breathlessness, and chest tightness. Asthmatic airways are characterized by chronic inflammation, eosinophil infiltration, epithelial fibrosis, mucus hyperproduction, and goblet cell hyperplasia [20].

It is considered as chronic allergic inflammatory disease, mostly mediated by a Th2 response, but an initial Th1-type immune response seems to be the trigger for the subsequent Th2-type response [82]. Thus, Th2 hyperactivation leads to persistent airway inflammation and the occurring of asthma phenotype [38].

Emerging evidences suggest that activation of autophagy is associated with reduced lung function in asthmatic patients. In particular electron microscopy analysis of fibroblast and epithelial cells from asthmatic patients showed increased autophagic hallmarks "such as double membrane autophagosomes" compared to healthy patients [75]. Unfortunately, at present, the role of autophagy in asthma is still unclear.

A recent study demonstrated that two Single Nucleotide Polymorphisms (SNPs), namely rs12201458 and rs510432 were associated with childhood asthma. In particular rs510432 localises at the promoter of ATG5 gene and could increase its expression in nasal epithelium of acute asthmatics compared to stable asthmatics and non-asthmatic patients [58]. Another intronic SNP variant (rs12212740) in ATG5 gene was also shown to be associated with pre-bronchodilator forced expiratory volume in 1 second (FEV1) in asthmatic patients [75].

ATG5 is an essential player in the initiation of autophagy, but its role in asthma pathogenesis is controversial. On one hand ATG5 could help viral elimination through the activation of Xenophagy, and on the other hand it negatively regulates the antiviral properties of type I interferon (IFN) inhibiting innate anti-virus immune responses [36, 90]. Together with these findings, lungs from conditional *Atg7* knockout mice manifest hyper-responsiveness to cholinergic stimuli, which is a common sign of asthma and chronic inflammatory diseases [31]. Asthma severity has been directly correlated with the level of autophagic response in the sputum granulocytes, peripheral blood cells and peripheral blood eosinophils of severe and non-severe asthmatic patients [5].

Autophagy is also involved in the maintenance of intracellular ROS homeostasis, and it has been well established that oxidative stress is associated with asthma so that exhaled levels of hydrogen peroxide ($H_2O_2$) and nitric oxide (NO) are currently used as predictors of asthma severity [68].

Chronic asthma is characterized by excessive ECM deposition and proliferation of myofibroblasts, leading to fibrosis in the airway wall [79]. The accumulation of fibrotic tissue is mostly due to the production of collagen A1 and fibronectin by the primary human airway smooth muscle through a mechanism autophagy-dependent that involves the TGFβ1. This response is reverted by the silencing of the major key autophagy-inducing gene Atg5 and Atg7 [104].

As already mentioned, asthma is a pathology mostly driven by Th2-type cytokines. Among them, IL-13 is extensively produced in activated $CD4^+$ Th2 lymphocytes and is overexpressed in the airway epithelium of asthmatic patients [47]. Here, IL-13 is thought to be responsible for epithelial hypertrophy, mucus hypersecretion, adventitial fibrosis and goblet cell hyperplasia [111]. It directly induces hypersecretion of mucin 5AC, oligomeric mucus/gel forming (MUC5AC) in airway epithelial cell and oxidant stress through a mechanism that is autophagy-dependent, as demonstrated *in vitro* by depletion of ATG5 or ATG14 in primary human tracheal-bronchial epithelial cells [15].

Autophagy might be involved in the pathophysiology of Alternaria (ALT-E)-associated asthma. ALT-E is an outdoor allergen able to activate autophagy, which in turn stimulates epithelial cells to release IL-18 [67]. This latter when produced is able to stimulate Th2 differentiation from naïve $CD4^+$ T-cells and IFN–γ production by Th1 cells. IL-18 level in serum of asthmatic patients might reflect the degree of disease exacerbation [94].

## 6 Cystic Fibrosis (CF)

Cystic Fibrosis (CF) is one of the most common lethal genetic diseases in Caucasian population. It is an autosomal recessive disease caused by mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Approximately 1 out of 20 Caucasians are carriers for mutation in this gene. Up to date over 2000 types of different mutations have been discovered and classified according to the degree of functional CFTR protein (http://www.genet.sickkids.on.ca/StatisticsPage.html; [27]). Among these, the most common one is the F508del-CFTR. Approximately 90 % of CF patients have at least one F508del-CFTR allele, and about 70 % are homozygous for it.

The CFTR channel is located at the apical surface of epithelial cells and it is deputized to move out $Cl^-$ from the cell. $Na^+$ passes through the membranes passively, increasing the movement of water by osmosis. Loss of functional CFTR expression is thought to alter this homeostatic balance through the epithelial layer, leading to net volume depletion of mucus, increased viscosity, and ineffective bacterial clearance [43, 78]. Recurrent pulmonary infections in turn induce an increased inflammatory response and signalling, thus starting a vicious cycle of mucus retention, infection, and inflammation. Since the CFTR is localized in many organs, CF symptoms could go from malabsorption at pancreatic level and gastrointestinal obstruction to male infertility and liver disease. Nevertheless, the main cause of

death remains persistent and untreatable pulmonary *Pseudomonas aeruginosa* infection.

Several recent studies have demonstrated an impairment of autophagy in CF. In fact, in epithelial cells, mutated/unfunctional CFTR causes increased ROS production with consequent increase in tissue transglutaminase type 2 (TG2) levels. TG2, in turn, leads to crosslinking of several targets including Beclin 1 [54, 57]. Beclin 1 interactome displaces from the Endoplasmic Reticulum (ER) leading to the sequestration of class III-phosphoinositide 3-kinase (PI3K) complex, accumulation of p62 with consequent inhibition of autophagosomes formation. The resulting accumulation of aggresomes leads to proteasome overload and may promote the accumulation of mutated CFTR in intracellular aggregates [54]. Restoration of Beclin 1 activity, depletion of p62 by genetic manipulation or treatment with autophagy-stimulatory proteostasis regulators, such as cystamine, functionally rescue the CFTR mutated protein at the apical surface of epithelial cells both *in vitro* and *in vivo* [54].

Heme oxygenases are enzymes involved in the catabolism of the heme ring to generate carbon monoxide, biliverdin-IXα, and ferrous iron. The inducible isoform Heme oxygenase-1 (HO-1) is activated in response to stress such as oxidative stress, hypoxia, heavy metals exposure and cytokines. HO-1, together with its enzymatic products, is able to inhibit apoptosis and related cell death pathways, conferring tissue protection in case of lung or vascular injury [66]. HO-1 could represent the link between CF and impaired autophagy since its expression is increased in human bronchial CF cells. This increase has been associated either to the reduction of apoptosis/injury during *P. aeruginosa* challenge either to the expression of inflammatory mediators [109]. Other evidences suggesting the cytoprotective role of HO-1 in CF showed that Lipopolysaccharide (LPS)-challenged CF macrophages fail to compartmentalize HO-1 to the cell surface and this mechanism seems to be dependent on the reduction in Caveolin-1 (CAV-1) expression [107]. In fact, when HO-1 localises at the plasma membrane, is able to form a complex with CAV-1, which in turn binds and detaches MyD88 from its complex with TLR4 thus terminating the cell death signal [99].

Autophagic clearance of bacteria (so-called Xenophagy) could also be impaired in case of disease, inducing increased bacterial infection that is one of the most frequent injuries in CF patients [90]. In fact it has been demonstrated that *Burkholderia cenocepacia* has the capacity to survive in F508del-CFTR macrophages since immediately after the engulfment, the bacteria resides on LC3-positive vacuoles that appear as arrested autophagosomes [98]. This capacity is directly correlated to the levels of p62, so that its depletion leads not only to a decreased bacterial survival in macrophages but also to the release of Beclin 1 from aggresomes allowing its recruitment to the *B. cenocepacia* vacuole and bacterial clearance via autophagy [2]. *B. cenocepacia* represents a serious threat for CF patients since the infection results in persistent lung inflammation and the bacteria are resistant to most of all available antibiotics [1].

Similar findings showed that pharmacological or molecular inhibition of autophagy reduces the clearance of intracellular *Pseudomonas aeruginosa* in vitro [37].

Treatment of CF mice with the mTOR inhibitor Rapamycin decreases bacterial burden in the lungs and drastically reduces signs of lung inflammation [1].

In a normal situation, autophagy can help not only removing polyubiquitinated protein but also controlling bacteria clearance; for these reasons novel strategies aimed at restoring autophagy are emerging as promising therapeutic approaches for CF patients [56].

## 7 Alpha-1-Antitrypsin Deficiency (AATD)

AATD is a hereditary disorder characterized by a low serum level of alpha-1-antitrypsin (AAT), a 52 kDa serine protease inhibitor, member of the serpin family [29]. AAT is essentially synthetized in the liver and secreted into the bloodstream, where it controls tissue degradation by the enzyme neutrophil elastase. The deficiency in AAT is associated with liver and lung disease due to the loss of anti-inflammatory and antiproteolytic functions. The majority of patients with AAT deficiency are homozygotes for a missense mutation ("PiZ mutation": lysine replaces glutamic acid at position 342) that alters protein folding. Mutant AAT molecules polymerize and aggregate in the ER of hepatocytes, forming large intrahepatocytic globules, the characteristic features of this disease. The proteasome is responsible for degrading the soluble form of ATT by means of ER-associated degradation while autophagy is involved in disposal of insoluble ATT polymers and aggregates [74]. In fact, a significant accumulation of autophagic vacuoles was found *in vitro* and *in vivo* in liver cells from AATD patients as well as in PiZ mouse model [96, 97]. Whereas in absence of autophagy the degradation of AAT was retarded [39]. Moreover, it has been demonstrated that the stimulation of autophagy by carbamazepine or rapamycin treatment or by liver-directed gene transfer of transcription factor EB (TFEB), a gene regulating lysosomal function and autophagy [89], reduce the hepatic amount of AAT as well as the hepatic fibrosis in mice expressing mutant AAT [28, 41, 71]. Although these results should be corroborated, altogether indicate that autophagy exerts a protective role in AATD and open a real possibility to treat AATD with pro-autophagic molecules.

## 8 Pulmonary Hypertension (PH)

Pulmonary hypertension (PH) was first identified in 1891 by Ernst von Romberg. PH is a severe and progressive disease that consists in increased blood pressure of lung vasculature and, often, can be a complication of chronic lung disease [88].

Since 2008 the pathology has been classified, by the World Health Organization (WHO), in five groups on the basis of mechanisms underlying the pathogenesis of the multiple types of PH.

The role of autophagy in pulmonary hypertension has mainly been described in correlation with pulmonary arterial hypertension (PAH), WHO Group I.

Little is known about the aetiology of PH, one of the most frequent genetic mutations causing idiopathic inherited form of PH is found in the gene encoding bone morphogenetic protein (BMP) receptor type-II (BMPR2).

In PAH, the pulmonary artery smooth muscle cells (PASMCs) proliferate excessively and are resistant to apoptosis. Chloroquine, a known inhibitor of autophagy flux, has been described as a drug preventing experimental PAH progression. The induction of PAH, by monocrotaline, in rat is associated with increased autophagy and decreased BMPR2 protein expression. The inhibition of autophagy by chloroquine ameliorates the level of BMPR2, inhibits the proliferation and stimulates apoptosis of rat PASMCs [52]. A recent publication [50] confirms that the inhibition of autophagy, by overexpressing mTOR, is a promising therapeutic strategy against PAH.

However, the role of autophagy in PH is still unclear and controversial, in fact, its protective role has been described in the initial phase of the pathogenesis of PH. Histochemical analysis of samples obtained from human PH lungs and mouse exposed to chronic hypoxia, showed an increase in the lipidated form of LC3 and in Egr-1, which regulates LC3 expression. Moreover, *LC3*$^{-/-}$ or *Egr-1*$^{-/-}$, but not *Beclin 1*$^{+/-}$ mice are more susceptible to PH and *in vitro* LC3 knockdown cells showed an increase of hypoxic cell proliferation, suggesting a role for LC3 in the adaptation during vascular remodelling under hypoxia [49].

## 9 Autophagy in the Etiology of Lung Cancer

In most organs, including the lung, autophagy robustly counteracts malignant transformation, *i.e.*, the conversion of a healthy cell into a (pre-)neoplastic cell, and several mechanisms related to the ability of autophagy to preserve cellular or organismal homeostasis account for such a pronounced oncosuppressive activity [19]. Indeed, besides being required for oncogene-induced senescence and anticancer immunosurveillance (see above) [112], autophagy promotes the maintenance of genomic integrity by multiple mechanisms [25]. First, it mediates the degradation of damaged mitochondria, which are prone to overproduce genotoxic ROS and other redox active entities of endogenous and exogenous origin [22]. Second, proficient autophagic responses appear to be required for optimal DNA damage responses [59]. Third, autophagy is involved in the disposal of potentially oncogenic retrotransposons and micronuclei [80]. Moreover, autophagy generally mediates anti-inflammatory effects, and chronic inflammation is known to accelerate oncogenesis (at least in some tissues, including the lung) [14]. Finally, it has been proposed that autophagy is required for the preservation of normal tissue architecture, in particular at the level of the stem-cell compartment [23]. Although little is known on the deregulation of stem cells in pulmonary carcinogenesis, it cannot be excluded that autophagic defects may promote malignant transformation in the lung also via this mechanism [69]. Conversely, the ability of autophagy to preserve genomic and redox homeostasis seems very relevant in the context of lung tumorigenesis, which in a significant

proportion of cases is associated with tobacco smoking or exposure to environmental nanoparticles like asbestos crystals [65]. Indeed, the oncogenic effects of both smoking and asbestos have been linked to their ability to cause ROS overgeneration along with genetic/genomic defects and chronic inflammatory responses [12]. All these effects are limited, at least to some extent, by proficient autophagic responses.

Irrespective of the precise mechanisms whereby autophagy counteracts malignant transformation in the lung, various genetic interventions aimed at specifically disabling autophagy in the lungs have been shown to promote malignant transformation driven by several oncogenes, including mutated B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) [91], epidermal growth factor receptor (*EGFR*) [100], Kirsten rat sarcoma viral oncogene homolog (*KRAS*) [24, 77]. Intriguingly enough, in one of these models, accelerated oncogenesis caused by the lung-specific inactivation of ATG5 was linked to increased tumor-infiltration by immunosuppressive $CD4^+CD25^+FOXP3^+$ regulatory T cells [77]. Moreover, the concomitant bi-allelic inactivation of serine/threonine kinase 11 (STK11, best known as LKB1) and phosphatase and tensin homolog (PTEN), two tumor suppressor genes that inhibit autophagy [34, 87], has been shown to cause the formation of pulmonary squamous cell carcinomas that express high levels of the immunosuppressive molecule CD274 (best known as PD-L1) [102]. These latter observations strongly corroborate the notion that autophagy mediates not only cell-intrinsic, but also cell-extrinsic oncosuppression.

## *9.1 Autophagy in the Progression of Lung Cancer*

The capacity of autophagy to preserve cellular homeostasis is beneficial to healthy cells, but also beneficial to transformed cells. This implies that autophagy often (but not always) promotes tumor progression, i.e., the growth and evolution of a transformed cells into an ever more malignant cancer [62]. Indeed, malignant cells are often exposed to relatively adverse microenvironmental conditions, including a shortage of nutrients and oxygen (especially in poorly vascularized tumor areas), and autophagy is instrumental for these cells (as it is for their non-transformed counterparts) to cope with stress and proliferate. Along similar lines, the ability of autophagy to preserve stemness is beneficial for the host when it preserves normal tissue architecture, but detrimental when it sustains the malignant stem-cell compartment. Finally, autophagy supports the survival of malignant cells in key step of tumor progression, the so-called "epithelial-to-mesenchymal transition" (EMT). In this context, epithelial cancer cells "initially growing *in situ*" physically detach from ECM and become able to colonize surrounding tissues as well as distant organs. The EMT is required for all malignancies to become locally and distantly invasive, and critically relies on proficient autophagic responses [4]. In the presence of autophagic defects or pharmacological inhibitors of autophagy, indeed, malignant cells undergoing the EMT and detaching from the ECM, succumb to a form of regulated cell death often referred to as "anoikis" [73].

Corroborating these observations, the genetic and/or pharmacological inhibition of the autophagic machinery in established tumors has been shown to accelerate disease progression in various models of pulmonary oncogenesis, including (but not limited to) *BRAF*- and *KRAS*-driven tumorigenesis [24, 77, 91].

## *9.2 Autophagy in the Treatment of Lung Cancer*

Autophagy provides malignant cells with an increased resistance to various perturbations of homeostasis, including the lack of nutrient and oxygen that cancer cells normally experience in poorly vascularized tumor areas, as well as the presence of xenobiotics like chemotherapeutic agents and physical stress conditions like irradiation. An abundant amount of literature demonstrates indeed that chemical inhibitors of autophagy as well as genetic interventions that compromise autophagic responses accelerate (rather than inhibit) the demise of malignant cells exposed to a wide panel of chemotherapeutics or to irradiation, both *in vitro* and *in vivo*. These observations provided a strong rationale to the development of combinatorial therapeutic strategies involving chemo- or radiotherapy given in combination with an inhibitor of autophagy [19].

Clinical grade highly specific chemical inhibitors of autophagy, however, have not yet been developed, and currently available molecules that can be used in the clinic, like chloroquine (a widely employed antimalarial agent) often operate as lysosomal inhibitors, i.e., they target several processes other than autophagy [83]. Moreover, concerns have been raised that inhibiting autophagy at the whole-body level may *de facto* favor malignant transformation in healthy tissues, reflecting the prominent oncosuppressive functions of autophagy in physiological conditions [51]. Finally, recent data highlight the differential role of autophagy in cancer therapy in immunocompromised *versus* immunocompetent hosts [44]. In this setting, the response to radiotherapy of human non-small cell lung carcinoma (NSCLC) or murine colorectal carcinoma (CRC) cells xenografted in nude mice was significantly improved when cells were rendered autophagy-deficient by the stable depletion of ATG5 or Beclin 1 [44]. However, when murine CRC cells were implanted in immunocompetent syngeneic mice, the stable knockdown of ATG5 compromised the therapeutic activity of irradiation, a defect that could be restored (at least in part) by the intratumoral administration of a chemical inhibitor of extracellular ATPases [44]. These findings demonstrate that inhibiting autophagy in immunocompetent hosts may prevent the elicitation of a therapeutically relevant immune response against dying cancer cells.

In summary, although autophagy generally (but not always) promote the progression of pulmonary malignancies and increases the resistance of lung cancer cells to chemo- and radiotherapeutic regimens, additional experiments are required to understand whether combinatorial treatments involving autophagy inhibitors constitute a clinically viable approach against pulmonary neoplasms. Similarly, further work is needed to clarify whether biomarkers of autophagy such as the expres-

sion levels of Beclin 1 or the lipidation of LC3 have a positive or negative prognostic/predictive value in patients with lung cancer, as preliminary results are rather controversial [40, 110].

## 10 Conclusions

Abundant evidences indicate that autophagy actively participates in a wide range of cellular responses to both physiologic- and pathologic-related events in the diverse tissues and cell types that constitute the lung system. Nevertheless, much is yet to be learnt about its biological relevance, functional targets, and role in development and disease. As described in this chapter, lungs are the first line of defence against several insults and associated diseases are growing both in number and chronicisation. A clear deregulation of the autophagic machinery has been highlighted in most of the lung diseases, suggesting that this process mainly exerts a defensive role. However, in some pathological contexts, it has been reported that the activation of the autophagic process contributes to damage. As a consequence, a detailed knowledge of the molecular mechanisms at the basis of autophagy in lung pathologies is required for the development of novel diagnostic tools and promising therapeutic strategies.

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