Chapter 39
Chronic Obstructive Pulmonary Disease and Autophagy

Xiaoxi Lv, Ke Li, and Zhuowei Hu

Abstract Chronic Obstructive Pulmonary Disease (COPD) is a classical chronic respiratory disease with the pathological changes involving the bronchi and alveoli. Many of the risk factors of COPD can induce autophagy in different kinds of cells in lung tissue including alveolar epithelial cells, broncho epithelial cells, and fibroblasts. Over-activation of autophagy may cause emphysema by inducing autophagic cell death. However, the bronchitis and fibrosis may be mainly caused by autophagic flux blocking. Thus, understanding the role of autophagy in the pathogenesis of COPD is important for the anti-COPD drug development.

Keywords COPD · Smoking · Emphysema · Bronchitis

39.1 Chronic Obstructive Pulmonary Disease

COPD is characterized by combination of persistent airflow limitation, small airway fibrosis, endobronchial goblet hyperplasia, and emphysema. The incidence of COPD is increasing year by year, and by 2020, COPD is expected to be the third most deadly disease in the world. The early stage of COPD is mainly characterized by chronic bronchitis. At this time, the pathophysiological changes are limited to small airways. The dynamic lung compliance reflecting lung tissue elastic resistance and small airway resistance declines. As the disease progresses, it gradually affects the airway, leading to pulmonary ventilatory dysfunction. The death of lung epithelial cells causes the damage of capillaries around the alveoli and results in a large decrease in blood flow among the alveoli. There are also some regions of the lung with normal blood perfusion, but the alveolar ventilation is poor and cannot support the gas exchange. Ventilation dysfunction can cause hypoxia and carbon dioxide retention,

X. Lv · Z. Hu (✉)
Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China
e-mail: huzhuowei@imm.ac.cn

K. Li
Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

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hypoxemia and hypercapnia, and eventually respiratory failure. The investigation of the pathogenesis of COPD proves that smoking is the primary pathogenic factor of COPD, especially in elderly male who smoke for a long time. In addition, the deletion of α1-antitrypsin is also an important cause of the onset of COPD. There are currently no clinical used drugs to reverse the lung function in COPD patients, but drugs and other treatments can significantly improve the life quality of patients. A study found the enhanced expression of autophagy markers in lung tissue of COPD patients, suggesting the role of autophagy in the pathogenesis of COPD. Thus, the studies which focus on the relationship between autophagy and the pathogenesis of COPD, especially smoking, α1-Antitrypsin, muscle atrophy, and autophagy are important (Alexandra et al. 2018).

39.2 Smoking and Autophagy

The study found that the number of autophagosomes in lung tissue of patients with COPD is increased, as well as the expression of LC3B-II, Atg4, Atg5-Atg12 complex, and Atg7. The chronic smoking model is the most commonly used COPD animal model in preclinical studies. The autophagy of lung is increased in C57BL/6 mice after 12 weeks of smoke exposure. The lung tissue sections showed an increase in the number of autophagosomes, and the expressions of multiple autophagy-related proteins are also enhanced. Knockdown of genes LC3B or beclin 1 had an inhibitory effect on smoke-induced COPD in mice, while autophagy signal deletion can inhibit cell death caused by Cigarette Smoke Extract (CSE). These evidences suggest that the autophagy signal is activated during the smoking process and the number of autophagosomes is increased. However, there are still different finding on the autophagy activity changes caused by smoking. After stimulation of different cells with bafilomycin A1 and CSE, the transformation of LC3B-I and LC3B-II illustrated that autophagic flux may be activated or inhibited by CSE in different cells (Chen et al. 2010).

Early studies generally suggested that oxidative stress and Reactive Oxygen Species (ROS) are the main causes of smoking-induced autophagy. ROS, as a signaling molecule that induces autophagy, activates a variety of autophagy pathways. Cigarette Smoke (CS) can promote the pro-oxidative state of epithelial cells, which in turn leads to oxidative stress and induces autophagy. CS-induced autophagy can be inhibited using the antioxidant *N*-acetylcysteine or NADPH oxidase inhibitor. Starvation is the most common method to activate autophagy with increased intracellular ROS levels during starvation, and this change can be inhibited by antioxidants. Thus, oxidative stress is not a specific agonist of CS-induced autophagy, but a universal autophagy-inducing biological mechanism. Furthermore, ROS-induced cell death can also be inhibited by knocking down autophagy-related genes (beclin 1, Atg4, Atg5, or Atg7), suggesting that ROS may mediate cell death by over-activation of autophagy (Bi et al. 2019).

The response protein early growth response-1(Egr-1) is an important factor regulating the transcription of LC3B. The expression of Egr-1 is up-regulated under stress, and the highly expressed Egr-1 is involved in cell apoptosis and inflammatory cascade. When CSE stimulates epithelial cells, Egr-1 rapidly binds to the LC3B promoter and induces LC3B transcription. Knockdown of Egr-1 can inhibit the autophagy induced by CS exposure. Interestingly, Egr-$1^{-/-}$ mice are resistant to autophagy activation and apoptosis induced by CS exposure and not sensitive to emphysema caused by CS exposure. Moreover, knocking out Egr-1 causes a physiological increase in the alveolar space of mice compared to wild-type mice. However, these effects may be caused by other biological effects regulated by Egr-1, including regulation of inflammatory cell infiltration, apoptosis signaling pathways (Morse and Rosas 2014).

The activity of autophagy may be related and resulting in dysregulation of ubiquitin-proteasome system activity during the pathogenesis of COPD, and numerous ubiquitinated proteins aggregate in the lungs of COPD patients mediated by CS exposure. Under normal conditions, intracellular ubiquitinated protein aggregates are often cleared by the autophagy pathway, but the number of intracellular ubiquitinated protein aggregates is increased at the onset of COPD, which suggests that impaired autophagy activity may be involved in the pathological changes of COPD. CS can promote the accumulation of p62 in cells, thus CS will cause damaged autophagic flux in the cells, and the accumulation of p62 can also be detected in lung tissue of COPD patients. The use of autophagy inducer carbamazepine not only reduces the number of intracellular protein aggregates induced by CS, but also inhibits CS-induced inflammatory responses and apoptosis.

In summary, autophagy is involved in the whole process of CSE-induced COPD pathogenesis, but the biological effects mediated by autophagy differ in different cells or at different stages of the disease (Haspel and Choi 2011). Using different models to study autophagy and the pathogenesis of COPD may lead to different conclusions. It is known that excessive activation of autophagy signals and inhibition of autophagic flux are important links in the pathogenesis of COPD. Over-activation of autophagy induced by ROS in epithelial cells causes cell apoptosis, and blocking autophagic flux may result in many misfolded proteins or ubiquitinated protein aggregates in cells. Autophagy activation and inhibition may occur in different regions in the same lung with COPD, so only normal autophagy function will not cause pathological changes (Fig. 39.1). CS contains more than 4700 components, including carbon monoxide, heavy metals, acetaldehyde, aromatic hydrocarbons, oxygen-free radicals, etc., which together regulate the onset of COPD. This caused great trouble for the study of CS-induced COPD. Current experimental methods are difficult to determine exactly which components of CS regulate autophagy activity. In addition, epidemiological data show that non-smokers can also have the same symptoms and pathological changes as CS-induced emphysema or COPD, suggesting that CS is not an absolute factor in the induction of COPD.

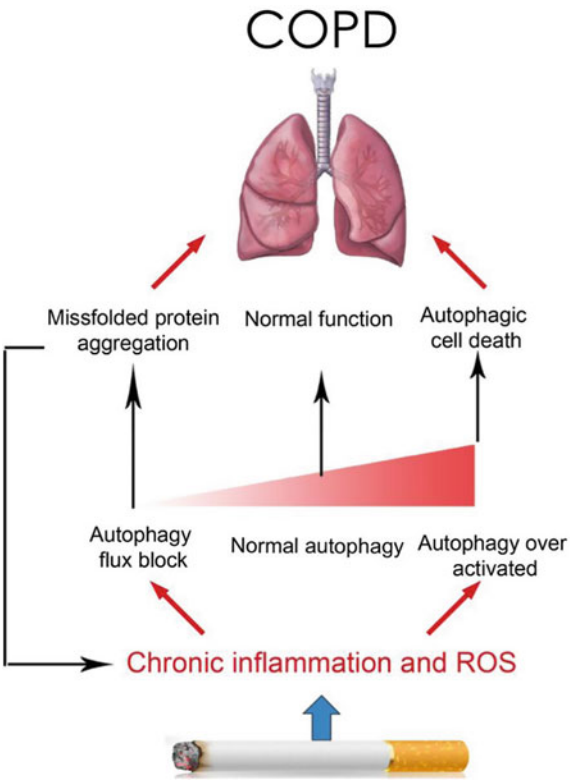

Fig. 39.1 Smoking regulates autophagy and promotes COPD. Long-term smoking can cause chronic inflammation and oxidative stress damage in the lung tissues. Oxidative stress and other factors cause excessive activation of autophagic flux in lung epithelial cells, that will induce autophagic death of lung cells, leading to emphysema. When the autophagy flux is blocked by chronic inflammation, the misfolded proteins and damaged organelles in the epithelial cells are accumulated, that accelerating the development of chronic inflammation

39.3 Autophagy Participates in Airway Remodeling in COPD

Airway remodeling is one of the characteristics of COPD, but the biological mechanism of its occurrence has not been fully determined. The pathological manifestations of airway remodeling are squamous changes in the pseudostratified ciliated columnar epithelium, cilia reduction, loss, and motor dysfunction, at the same time, the goblet cells in the airway enhancement, smooth muscle and fibrous connective tissue hyperplasia, and infiltration of a large number of inflammatory cells. Eventually, bronchial fibrosis may appear and emphysema occurs in the abnormal distal end of the bronchus. Smoking is the most important factor leading to COPD, which can produce a large amount of reactive oxygen species leading to the conversion of type I LCB to type II and the formation of autophagosomes in epithelial cells. Reactive oxygen species activates LC3B by increasing phosphorylation of JNK, and inhibition of reactive oxygen species can inhibit autophagy induced by smoking in airway epithelial cells. In another trending factor in COPD, neutrophil elastase can damage the bronchial epithelium and promote the progression of COPD. Neutrophil elastase

increases the level of PGF by Egr-1, thereby increasing the level of autophagosomes in damaged cells. Therefore, PGF in the serum of COPD patients can be used as a new drug target for autophagy treatment and drug discovery. In addition to smoking, environmental pollution is also an important cause of COPD. PM2.5 is a particle that can enter the respiratory tract with airflow and enter the circulatory system through the respiratory tract. Exposure of bronchial epithelial cells to PM2.5 increased the expression of LC3B, Beclin1, and VEGFA in cells, as well as the ratio of LC3BII/LC3BI and the number of autophagosomes (Zhu et al. 2018). 3-MA can inhibit the increase of VEGFA level caused by PM2.5, which proves that autophagy plays an important role in the VEGFA level enhancement caused by PM2.5. It is also involved in chronic inflammation and vascular remodeling caused by VEGFA. IL-13 is an important Th2 type cytokine involved in airway remodeling in the pathogenesis of COPD. IL-13-stimulated bronchial epithelial cells secrete elevated levels of MUC5AC and LC3B, knocking down Atg5 reduces secretion of MUC5AC in epithelial cells caused by IL-13, suggesting that IL-13 can induce autophagy to regulate airway epithelial cells secrete function.

39.4 Autophagy Regulates Apoptosis of COPD

Apoptosis of epithelial cells is an important mechanism of the pathogenesis of COPD, and apoptosis occurs in vascular endothelial cells, stromal cells, and inflammatory cells. The expression of apoptosis-related proteins caspase-3, Bax and Bad in lung tissue of COPD patients are increased, while the expression of anti-apoptotic protein Bcl-2 was unchanged, and these apoptosis-related proteins did not increase in the lungs of non-COPD smokers. There is evidence that even if smoking COPD patients quit smoking, their lung cells will still undergo apoptosis, so oxidative stress caused by smoking is not necessarily for lung cell apoptosis in COPD patients. Other causes that have been shown to induce apoptosis in COPD include protease/antiprotease imbalance and excessive inflammatory response.

Evidence suggests that activation of caspase-3 is only present in the lungs of patients with severe COPD (Global initiative for chronic Obstructive Lung Disease [GOLD] 4) but not in mild patient tissues (GOLD 0-2). However, all patients with COPD grade (GOLD 0-4) had elevated levels of autophagy markers in the lung tissue, including the expression of LC3B-II and the number of autophagosomes. This indicates that the change in autophagy activity during chronic exposure to CS is earlier than apoptosis. LC3B is the most classical marker protein of autophagy involved in CSE-induced epithelial cell apoptosis (Li et al. 2017). The number of apoptotic cells in the lungs of $LC3B^{-/-}$ mice was significantly lower than wild-type mice after CS exposure. CSE can induce apoptosis by inducing Fas-dependent Death-Inducing Signaling Complex (DISC) and activation of caspase-8. LC3B can form a complex with Fas and other death receptors in epithelial cells. LC3B is able to inhibit CSE-induced DISC formation. The LC3B-Fas complex formation is dependent on caveolin-1. In normal cells, caveolin-1 binds LC3B to form a trimer with Fas, thereby

inhibiting the formation of DISC by Fas. At the same time, LC3B can promote the dissociation of caveolin-1 and Fas. Knockdown of LC3B can enhance the interaction between caveolin-1 and Fas and further inhibit DISC formation to maintain cell survival.

39.5 Autophagy Regulates Macrophage Function to Participate in the Pathogenesis of COPD

Macrophages isolated from the lungs of COPD patients have generally lost their ability to phagocytose pathogens, but still are capable of releasing large amounts of pro-inflammatory cytokines. These phenomena ultimately lead to susceptibility to exogenous bacteria and bronchial inflammation in COPD patients. Analysis of autophagic flux in alveolar macrophages from patients with COPD over a 10-year history of smoking showed that autophagy was blocked after smoking. The specific analysis results show that the blockage of autophagic flux in macrophages is due to the slow degradation of autophagosome. In addition, Mitochondrial damage in macrophages may be an important cause of immune function loss.

Autophagy blockade in patients with COPD is also one of the reasons for the release of pro-inflammatory cytokines. Alveolar macrophages in COPD patients can release large amounts of IL-1β, causing terminal bronchial inflammation. This biological effect is composed of the following two factors. (1) Autophagy dysfunction in macrophages leads to activation of caspase-1 dependent on the Toll receptor linker protein TRIF, thereby promoting the production of IL-1β. Activated caspase-1 is able to regulate the release of IL-1β by its cleavage effect. Macrophages of Atg16L1-deficient mice produce large amounts of IL-1β and IL-18 compared to wild-type mice. Atg16L1/TRIF double knockout mice lost the ability to produce IL-1β form macrophage due to the inability to produce caspase-1. (2) The IL-1β gene first forms proIL-1β after transcriptional translation, and then pro-IL-1β hydrolyzes a short peptide by a specific serine protease to form the active cytokine IL-1β. In many cases, exogenous stimuli promote synthesis of pro-IL-1β in macrophages. Pro-IL-1β is activated and released when macrophages receive a second signal stimulus. Macrophages store pro-IL-1β in lysosomes before their activation. When autophagy is activated, pro-IL-1β is degraded in lysosomes, which in turn negatively regulates the release of IL-1β. The use of 3-MA or wortmannin to inhibit autophagy activity of macrophages can promote the activation of inflammation and the release of pro-IL-1β.

39.6 α1-Antitrypsin and Autophagy

α1-Antitrypsin (AAT) level dysregulation is an important cause of COPD in non-smokers. Autophagy can mediate clearance of aggregated AAT intracellularly. The "PiZ mutation" is the most common type of mutation in the AAT gene, which can cause misfolding during AAT translation and cause it to accumulate in the endoplasmic reticulum. The PiZ-mutated AAT cannot be transported from the liver cells into the blood, resulting in a disorder of the protease/anti-protease balance in the local tissues of the lungs, which ultimately induces the formation of emphysema. The ratio of LC3B-II/LC3B-I in the lung tissue of patients with AAT mutations is increased, indicating the changed level of autophagy. And the accumulation of PiZ-AAT mutant protein in hepatocytes is co-localized with LC3B. In ex vivo experiment, autophagy inhibitors can inhibit the degradation of exogenous AAT proteins. The use of autophagy agonists can reverse the spontaneous liver pathological changes in PiZ-AAT mutant transgenic mice, indicating impaired autophagy activity in local parts under the condition of AAT mutation. In addition, PIZ-AAT mutant protein aggregates were also detected in alveolar lavage fluid and tissue samples from COPD patients. The accumulation of PiZ-AAT mutant protein in alveolar macrophages suggests autophagy dysfunction in macrophages during the pathogenesis of COPD.

39.7 COPD Muscle Atrophy and Autophagy

In addition to lung damage, patients with COPD usually have symptoms such as weight loss, malnutrition, limited exercise capacity, and skeletal muscle atrophy. Skeletal muscle atrophy and muscle dysfunction are important factors affecting the life quality of COPD patients. The overall performance decreased muscle tone and endurance. The decrease of muscle mass in patients is mainly due to the imbalance of protein synthesis and degradation. A large number of early studies have shown that muscle atrophy in COPD patients is mainly caused by abnormal activation of the ubiquitin-proteasome pathway, until the role of autophagy lysosomal pathway in COPD skeletal muscle atrophy is gradually revealed (Guo et al. 2013). Autophagy is a very conservative energy-regulating mechanism of eukaryotic cells. There is evidence that basal autophagy activity is critical for the stability of skeletal muscle cells, which can be responsible for removing accumulated proteins and mitochondria in cells, as well as inducing muscle production in lower limbs after denervation.

The response of skeletal muscle to autophagy is different from other tissues in the stress response. After starvation for 24 h, autophagy responses in most tissue cells were activated, and the autophagy activity gradually decreased after 48 h of starvation. Skeletal muscle cells can still produce a large number of autophagosomes after 48 h of starvation. Knocking out Atg7 in skeletal muscle cells can completely inhibit the production of autophagosomes, leading to the accumulation of abnormal mitochondria and polyubiquitinated proteins, oxidative stress, and dysplasia. These

biological reactions can eventually cause muscle fiber degeneration. Similarly, Atg7 knockout mice can exhibit myasthenia gravis and significant muscle wasting symptoms. Inhibition of autophagy in skeletal muscle cells accelerates muscle atrophy caused by fasting and denervation. Similar to Atg7 knockout mice, Atg5-deficient mice also showed significant muscle atrophy. Studies have shown that BCL2-related Nutrient-Deprivation Autophagy Factor-1 (Naf-1) is an important regulator of skeletal muscle cell homeostasis. Mice lacking Naf-1 also showed muscle weakness and decreased muscle tone. Naf-1 null mice developed progressive gradual decline at 2–3 months of age, and sudden death at 12 months of age. Histone Deacetylases (HDACs) in skeletal muscle cells are also involved in autophagy regulated by starvation. Skeletal muscle-specific loss of HDAC1 and HDAC2 can lead to perinatal lethality in some animals. HDAC1/2 knockout mice cause autophagic dysfunction after birth and develop muscle lesions and muscle production/degradation disorders.

Nowadays, the correlation between skeletal muscle physical movement and autophagy has been gradually elucidated. There is evidence that proper physical movement can induce skeletal muscle autophagy. Moreover, physical movement can inhibit the interaction of BCL2 with Beclin-1, thereby activating autophagy signals. BCL2 mutant (BCL2 AAA) mice were unable to induce skeletal muscle autophagy through exercise. BCL2 AAA mice showed significantly declined tolerance to exercise, glucose metabolism disorder after exercise, and loss of protection for poor glucose tolerance induced by high-fat diet. Tolerant protection. It can be seen that autophagy plays an important role in regulating the metabolic response induced by exercise.

The vast majority of patients with COPD have symptoms of decreased skeletal muscle mass, which was initially suspected to be due to impaired overall autophagy. However, the autophagy activity of lung tissue and skeletal muscle in patients with COPD does not increase or decrease at the same time. It is worth noting that the levels of LC3B-II, Beclin-1, and p62 in skeletal muscle samples of COPD patients were significantly increased compared with healthy people. However, these phenomena do not indicate the true activation of autophagy, but may also be the result of autophagic signal activation and autophagic flux blockade. At present, many studies suggest that smoking is an important factor in the increase of autophagy in patients with COPD. However, it is unclear whether the change in autophagy activity induced by smoking is systemic or lung tissue specificity, and which components in CS regulate autophagy activity of skeletal muscle remains unclear. It is worth noting that the pathological changes in lung tissue of patients with COPD are mainly caused by CS, but the changes in autophagy activity of skeletal muscle in patients are mostly caused by malnutrition, which may be another theory independent of autophagy. Autophagy is a complex biological reaction process that intersects with various biological effects, including apoptosis, proliferation, inflammation, etc. The biological effects of autophagy in different tissues of the same individual are also different. Therefore, it is currently difficult to correctly define the role of autophagy in skeletal muscle atrophy in COPD patients.

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