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Autophagy, Unfolded Protein Response and Lung Disease

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Abstract

Acute Respiratory Distress Syndrome is a severe disorder affecting thousands of individuals worldwide. The available medical countermeasures do not sufficiently suppress the unacceptable high mortality rates associated with those in need. Thus, intense efforts aim to delineate the function of the lung endothelium, so to deliver new therapeutic approaches against this disease. The present manuscript attempts to shed light on the interrelations between the unfolded protein response and autophagy towards lung disease, to deliver a new line of possible therapeutic approaches against the ferocious Acute Respiratory Distress Syndrome.

Keywords

Acute Lung Injury; COPD; Pulmonary Hypertension; Tuberculosis; Cystic Fibrosis; Endothelium

Autophagy in the UPR context

Autophagy is a well-orchestrated process, controlled by over 30 autophagy-related (ATG) genes [1]. It functions to remove the misfolded or aggregated proteins, to eliminate impaired organelles (i.e. mitochondria, peroxisomes), as well as to eradicate intracellular pathogens [2]. In addition, autophagy enhances cellular senescence, defends genome instability, and prevents necrosis [2].

Three different types of autophagy have been identified, namely the macro-autophagy, the micro-autophagy and the chaperone-mediated autophagy. Macro-autophagy is the most extensively studied type. Under normal physiological conditions, it helps to maintain the cellular homeostasis by degrading damaged or redundant organelles [3]. In stress conditions, including nutrient or energy deprivation, it degrades the cytoplasmic materials into metabolites to employ them towards biosynthesis, energy production, or cell survival [4].

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Autophagy is primarily a cellular protective mechanism. However, excessive autophagy has been associated with various aspects of human pathology, including lung disease [5]. It can be measured by evaluating the levels of autophagic flux, by detecting the lipid modified form of the microtubule-associated protein 1A/1B-light chain 3 (LC3), as well as the autophagosome. Increased levels of autophagosomes do not always reflect enhanced autophagy, but it may represent blocked autophagosome fusion or degradation. Hence, the assessment of autophagic flux is important to accurately measure enhanced autophagy [6].

Autophagy enhances endogenous defense mechanisms by upregulating molecular chaperones [7], and it is regulated by the mammalian target of rapamycin (mTOR) [8]. Deactivation of mTOR enhances autophagy [9], which in turn regulates the unfolded protein response, a sensor of endoplasmic reticulum (ER) stress fluctuations. ER is a complex cellular organelle involved in protein synthesis and folding [10], as well as changes in Ca^{2+} concentrations [11].

The ER lumen contains an array of chaperones and enzymes for processing of nascent proteins. Thus, it provides a unique oxidizing environment to support the formation of disulfide bond during protein folding [12]. ER disruption due to hypoxia, nutrient deprivation, redox changes [13], increased secretory load, and increased load of mutated proteins [14] cause ER disturbance, accumulation of misfolded or unfolded proteins in the ER lumen and ER stress. Adapting to those conditions requires the activation of a highly conserved intracellular response mechanism, namely the unfolded protein response (UPR) [15].

The UPR is consisted of three transmembrane receptors. Those sensors are the protein kinase RNA like ER kinase (PERK), the activating transcription factor 6 (ATF6), and the inositol-requiring enzyme-1 (IRE1) [16]. Those receptors are bound to BiP [17]. The higher affinity of BiP to misfolded or unfolded proteins causes its dissociation from the previously mentioned UPR branches to trigger downstream signaling processes [18, 19].

Inactive PERK is monomeric. Upon BiP release, it homodimerizes and consequently trans-autophosphorylates. Activated PERK mediates the phosphorylation of the α-subunit of eukaryotic initiation factor 2 (eIF2); which in turn reduces the protein load in the ER [20]. ATF6 is a type II transmembrane receptor with two isoforms in the mammalian genome, namely the ATF6α and ATF6β. ATF6α is involved in UPR gene expression [21]. After dissociation from BiP, ATF6 is transferred to the Golgi apparatus to be cleaved by site 1 protease (S1P) and site 2 protease (S2P). Those processes produce the active cytosolic p50 fragment, which migrates to the nucleus. That fragment activates the expression of genes which increase the ER folding capacity, and activate the endoplasmic reticulum-associated protein degradation pathway (ERAD) [21]. ATF6 activation has also been reported to regulate an array of miRNAs to ameliorate ER stress [22].

The type I ER transmembrane protein IRE1 accommodates two distinct domains in its cytoplasmic tail; a serine/threonine kinase domain and an endoribonuclease (RNase) domain [23]. IRE1 has two isoforms, namely the IRE1α and IRE1β. IRE1α is predominantly expressed in almost all tissues. IRE1β expression is limited only to the intestinal epithelial

cells [24] and the airway mucous cells [25]. IRE1, which is the most evolutionary conserved signaling branch of UPR [26]; it is closely associated with pro-survival pathways enabling the cells to adapt to stressful environments [27]. The release of IRE1a from BIP causes its dimerization and auto-phosphorylation, which in turn activates IRE1α [28]. It was also reported that IRE1α binds directly to unfolded proteins, and becomes activated in a similar fashion [29].

The activated IRE1α exerts RNase activity to cleave a single RNA encoding X-box binding protein 1 (XBP1). Those activities produce a potent transcriptional activator (XBP1s) [28]. XBP1s enters into the nucleus to induce the expression of several proteins that attenuate ER stress (i.e. ER-resident chaperone BiP, protein folding enzyme PDI, and translocation proteins) [30]. Furthermore, XBP1s augments the expression of the heat shock protein 40kda (DnaJ) and the ER-associated degradation system or ER-to-golgi transport components. Thus, it degrades unfolded proteins in the ER lumen [31, 32]. Recent observations suggests that Hsp90 inhibition induces the unfolded protein response in the lungs both *in vivo and in vitro* [33, 34], and that P53 might be involved in those effects [35–37].

A correlation between the unfolded protein response (UPR) and autophagy has been demonstrated in many studies. Several stimuli cause the accumulation of potentially toxic polypeptides in the ER lumen, and overload the capacity of the chaperone system. This condition eventually engenders ER stress, which triggers the activation of UPR to avoid cell damage and promote the protein folding capacity of ER [38]. Hence, it eliminates the impaired polypeptides through ERAD and ER stress-induced autophagy [39].

PERK activation and autophagy

The expression of autophagy-related proteins are significantly increased by the PERK-eIF2α-ATF4 pathway [40, 41]. Phosphorylation of eIF2α through ER stress-induced PERK activation causes the induction of ATF4. The latter protein binds to CHOP and consequently augments the transcription of various autophagy-related genes (e.g. Atg3, Atg7, and Atg12) [41].

The expanded polyglutamine 72 repeat (polyQ72) can induce autophagy through phosphorylation of PERK/eIF2α [42]. PolyQ72 resides in the cytoplasm and inhibits the translocation and degradation of ER proteins through ERAD system. A combination of these two effects causes ER stress; which accelerates the Atg5-Atg12-Atg16 complex conversion, a process which involves LC3-I to LC3-II. LC3-II formation is an important characteristic of autophagy [42]. The chalcone Xanthoangelol promotes the UPR induced-autophagy via activation of the JNK/c-Jun pathway. It upregulates the expression of several proteins involved in autophagy (e.g LC3-II, Beclin-1, and Atg5 [43]. Oxidized lipids also employ the JNK-dependent autophagy by triggering UPR, and further promote cell survival during oxidative stress [44].

IRE1α activation and autophagy

IRE1 initiates autophagy. Manganese (Mn) can induce IRE1 phosphorylation through UPR activation. Phosphorylated IRE1 is involved in JNK activation by producing the ASK1-TRAF2 complex [45]. JNK phosphorylates and activates c-Jun, upregulating Beclin 1 transcription [46]. Beclin1 exerts a crucial role in promoting autophagy through autophagosome formation. Hence, it serves as a molecular switch in the regulation of autophagy [47]. IRE1 induces autophagy via the IRE1-JNK pathway [48].

ATF6 activation and autophagy

Japanese encephalitis virus (JEV) induces autophagy in neuronal cells via ATF6 activation. The suppression of ATF6 by siRNA significantly decreased the levels of Atg3 and LC3-II [49]. The core protein of Hepatitis C virus promotes ATF6 activation and CHOP protein expression. The latter protein upregulates the transcription of LC3B by directly binding to its promoter region. Moreover, CHOP induces the expression of Atg5, promoting the autophagosome formation [50]. ATF6 activation is a pro-survival pathway for protecting the neuron after ischemic stroke, due to the elevation of autophagy in the neurons of ATF6-KI mice. The short-form of ATF6 (sATF6) upregulates LC3-II, downregulates p62, and activates mTOR in post ischemic brain [51].

Autophagy and UPR in chronic obstructive pulmonary disease (COPD).

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease that causes obstructed airflow from the lungs. Emphysema, inflammation, and fibrosis of the small airways have been associated with that disorder [52, 53]. Cigarette smoke (CS) is the most important risk factor of COPD [54]. The underlying molecular mechanism of COPD remains incompletely understood, and autophagy is considered a major mechanism which contributes to the pathogenesis of CS-induced COPD [55]. The lung tissues of COPD patients have been shown to express an elevated level of several autophagy markers; including LC3-II, Atg4, Atg5/12, and Atg7 as well as increased levels of autophagosomes [56]. Epithelial cell lining of alveoli and airways are the primary targets of inhaled CS. Autophagosome formation in the lung epithelial cells indicates the activation of autophagy pathway in COPD [57]. Aggrephagy [58] and mitophagy [59, 60] were observed in the epithelial cells of COPD patients. The activation of selective autophagy was associated with detrimental effects in lung epithelial cells.

Oxidative stress-induced growth inhibitor 1 (OSGIN1) is a link between CS-induced stress and elevated level of autophagy in the human airways epithelium [61]. OSGIN1 is an apoptotic regulator extensively upregulated in the smokers compared to the non-smokers. The increased expression of OSGIN1 causes apoptotic cell death through inducing the cytochrome C release from mitochondria [62]. CS induces the OSGIN1 expression in large and small airways epithelium, and this enhanced OSGIN1 upregulates autophagy through MAP1LC3B and SQSTM1 [61]. Moreover, suppression of the MAP1LC3B, PINK1, and Hdac6 protect from smoke-induced emphysema and impairment of mucociliary clearance [59, 63].

COPD is also linked to the particulate matter (PM) exposure. Chronic exposure to biomass fuel (BMF) and motor vehicle exhaust (MVE), which are the two major sources of PM emission, causes pulmonary inflammation, alveolar enlargement and airway remodeling [64]. Patients with pre-existing COPD are more susceptible to PM exposure than healthy individuals. Exposure to PM demonstrates a reduction in CCAAT/enhancer-binding protein alpha (C/EBPα) in human bronchial epithelial (HBE) cells. That triggers the anomalous expression of NADH dehydrogenase genes, and induces autophagy. Recuperation of lung damage by the autophagy inhibitor 3-MA; or the genetic suppression of Atg7 in PM-associated COPD dictates the deleterious effects of autophagy in COPD [65]. These studies suggest that autophagy in the lung epithelial cell lines contributes to the development of COPD.

Alveolar macrophages (AMs) are required for bacterial clearance from the alveolar surface and the prevention of the microbe-induced infection. The AMs of CS-induced COPD patients accumulate autophagosomes and p62. The reduced autophagy in AMs leads to impaired protein aggregate clearance, dysfunctional mitochondria, and defective delivery of bacteria to lysosomes (defective xenophagy). Those effects explain the recurrence of bacterial infection for smokers [66]. Furthermore, insufficient autophagy is also associated with the persistent inflammation in COPD. Leukotriene B4 (LTB4) produces inflammatory cytokines that makes a crucial contribution to chronic inflammation. In order to induce inflammatory pathway, LTB4 binds to its' highly specific LTB4 receptor 1 (BLT1) which is expressed primarily in leukocytes [67]. CS significantly increases the BLT1-positive AMs [68] that mediates inflammation through the LTB4/BLT1 pathway [69]. Inadequate autophagy of macrophages is linked to the LTB4-mediated inflammation in CS-induced COPD model. The BLT1 antagonist exerts anti-inflammatory activity by inducing autophagy in AMs, and was suggested to serve as a potential therapeutic approach for the COPD management.

The pathogenesis of CS-induced COPD is also associated with increased levels of reactive oxygen species (ROS) caused by CS-induced mitochondrial damage [60]. CS-induced mitophagy can be inhibited by the PTEN-induced kinase 1 (PINK1). Decreased levels of Parkin RBR E3 ubiquitin protein ligase (PARK2) expression has been observed in COPD lungs [60]. However, another study indicated that the PINK1-regulated mitophagy promotes necroptosis and cell death in lung epithelial cells, thereby contributing to COPD development [59]. PINK1 null mice were protected against mitochondrial dysfunction, airspace enlargement, and mucociliary clearance (MCC) during CS exposure. In humans, lung epithelial cells of COPD patients displayed increased levels of PINK1, suggesting that this pathway can be a therapeutic target for COPD [59].

Studies employing the mTOR inhibitor rapamycin demonstrated that although enhancing autophagy may be beneficial during CS-exposure, autophagy induction by rapamycin increases the number of apoptotic and inflammatory cells [70].

UPR is also associated with the pathogenesis of COPD. The process of protein folding and refolding produces reactive oxygen species (ROS) inside the ER lumen. Hence, a suitable redox environment is essential for the proper ER function. This redox environment is

maintained by different ER-resident enzymes; including the ER oxidoreductin-1 (Ero1) family [71]. Cigarette smoke induces ROS production, and affects protein oxidation in the ER lumen, at least in part by the protein disulfide isomerase (PDI) modulation [72]. Those events activate the UPR in normal human bronchial epithelial (NHBE) cells, mice lung epithelial 12 (MLE12) cells, alveolar type II cells and alveolar macrophages [72–74]. In addition, oxidative damage of different cellular proteins due to CS exposure compromises proteomic clearance, and results in the deposition of those aberrant proteins inside the cells [75].

The activation of UPR is rapid and cell type - dependent. Moreover, its effects depend on the duration of its induction. It was suggested that initial UPR induction is protective [73] while its chronic induction upregulates different pro-apoptotic protein including the CHOP and caspases [76–78]. Since production of ROS by CS is the underlying reason of UPR activation, scavengers of ROS demonstrate a promising effect in reducing UPR and consequently the CS-induced apoptosis in HBE cells [76]. Similarly, NHBE cells from COPD donors expressed increased level of the UPR markers, ATF4, XBP1 and CHOP. GPx-1 null mice shows increased expression of CS-induced markers and apoptosis [79]. CS can also upregulate the nuclear factor erythroid 2-related factor 2 (Nrf2). This protein heterodimerizes to induce genes involved in the protection of lung cells against oxidative stress. This phenomenon occur after the exposure of CS in human airway epithelial cells [73].

CS-induced UPR activation causes the impairment of efferocytosis. This is a process by which apoptotic cells are removed in the alveolar macrophages. CS suppresses the efferocytosis by activating RhoA in macrophages both *in vivo* and *in vitro* [80]. RhoA is a key element of the cytoskeletal integrity [81–83]. PERK mediates the suppression of efferocytosis and PERK inhibitors successfully reverse RhoA activity to restore efferocytosis in alveolar macrophages [84].

Interestingly, chaperone-mediated autophagy (CMA) exhibits a reduction of CS-induced UPR and the subsequent apoptosis. CMA is involved in the maintenance of homeostatic processes, and it is responsible for the selective degradation of proteins containing Lys-Phe-Gln-Arg-Gln (KFERQ) [85]. CS induces lysosome associated membrane protein 2A (LAMP2A) and CMA through Nrf2-dependent manner in HBE cells. Suppression of LAMP2A and CMA inhibition results in the enhancement of UPR and apoptosis. This effect can be reversed by the induction of the LAMP2A expression [86].

Autophagy and UPR in pulmonary hypertension (PH).

Pulmonary hypertension (PH) is a pathophysiological condition defined as an increase of mean pulmonary arterial pressure [87, 88]. The classification of PH was initially proposed in 1973, suggesting the primary and secondary PH. Later on a different set of categories were established to reflect more accurate pathophysiological mechanisms, hemodynamic characteristics and therapeutic approaches towards PH. The new clinical classification categorized PH in: 1) pulmonary arterial hypertension (PAH) 2) PH due to left heart disease 3) PH due to chronic lung disease and/or hypoxia 4) chronic thromboembolic PH 5) PH due

to unclear and/or multifactorial mechanisms. In the third, fourth and fifth international symposium held in 2003, 2008 and 2013 respectively, additional amendments have been adopted to improve the clarity and to include new information regarding the PH pathophysiology [89–91].

PAH is a fatal pulmonary disease that causes right ventricular failure and death. Although its etiology was previously unclear, scientists have made a significant progress in understanding the causes and development of PAH. PAH can be hereditary (HPAH), idiopathic (IPAH) or associated (APAH) with medical conditions associated to connective tissue diseases, HIV infection, portal hypertension, congenital heart disease, and chronic hemolytic anemia. Furthermore, mutations in bone morphogenic protein receptor type 2 gene (BMPR2), activin-like kinase type 1 (ALK1) and endoglin (ENG) are associated with HPAH [92].

Hypoxia is a common cause of PH, associated with significant structural remodeling in the pulmonary arteries. The resident vascular cells (i.e. endothelial cells, smooth muscle cells, and adventitial fibroblasts) alter their proliferation, matrix protein production, cytokine release and receptor expression. Moreover, hypoxia potentiates the inflammation in the vasculature and recruits progenitor cells to advance structural vascular remodeling and vasoconstriction of the pulmonary circulation [93].

Elevated level of LC3B were observed in PH lungs [94]. LC3B knockout mice have shown increased susceptibility to hypoxia-induced PH. LC3B knockdown cells were associated to increased hypoxic cell proliferation. Those effects suggested adaptive functions of LC3B during hypoxic vascular remodeling [94]. In prolonged hypoxic conditions, an increased ratio of the microtubule-associated protein 1 light chain (LC3)-II to LC3-I has been observed in pulmonary arterial endothelial cells. Inhibition of autophagy by 3-methyladenine and beclin-1 knockdown led to decreased autophagy and increased *in vitro* angiogenesis [95].

Fibroblast, smooth muscle and endothelial cell proliferation cause thickening of the small pulmonary arteries, resulting in increased pulmonary vascular resistance. Increased proliferation of pulmonary arterial smooth muscle cells (PASMCs) and their resistance to apoptosis are key characteristics of PAH [96]. Induction of PAH by monocrotaline resulted to higher level of autophagy markers (i.e. LC3B-II, Atg5, p62); as well as decreased BMPR2 expression. Inhibition of autophagy by chloroquine prevented the development of PAH, right ventricular hypertrophy, and vascular remodeling. It has also suppressed the cellular proliferation, increased apoptosis and induced BMPR2 expression in PASMCs [97]. Thus, chloroquine exerted beneficial effects in experimental PAH.

ER stress and UPR are associated with the pathogenesis of PAH. IRE1, ATF6 and PERK have been associated to the PAH development. XBP1, CHOP, eIF2α, BiP and Grp94 have been upregulated after four weeks of chronic hypoxia condition. Treatment with 4-phenylbutyric acid (4-PBA), a chemical chaperone and ER stress suppressor, remarkably reduced ER stress and attenuated the right ventricular hypertrophy and pulmonary artery remodeling in hypoxic mice [98, 99]. Moreover, the targeted inhibition of eIF2α [100],

IRE1α/XBP1 [101] and ATF6 [99] promoted apoptosis, inhibited proliferation, and reversed hypoxia-induced vascular remodeling.

Recent studies demonstrated that eIF2α facilitates the proliferation of PASMCs and vascular remodeling through autophagy [102]. In addition, mTOR signaling enhanced the proliferation of PASMCs and induced PH via mTOR/eIF2α pathway. The mTOR inhibitor rapamycin suppressed eIF2α activation and attenuated vascular remodeling in hypoxia-induced PH [100].

ATF6 has been shown to accelerate the development of PAH through the upregulation of Nogo-B, a crucial protein for maintaining the tubular structure of ER. Induction of Nogo-B disrupts the ER-mitochondria unit, and suppresses the mitochondria-dependent apoptosis in PASMCs. The rescue of PASMCs from apoptosis during ER stress accelerates the development of PAH via proliferation [103].

Role of autophagy and UPR in tuberculosis.

Pulmonary tuberculosis (TB) is a chronic lung infection caused by the *Mycobacterium tuberculosis* (Mtb) [104, 105]. Autophagy has a crucial role in the pathogenesis of tuberculosis. Induction of autophagy is a potential mechanism to eliminate intracellular pathogens [106], and inhibition of autophagy may serve as a potential survival strategy of bacterial pathogens like Mtb. The fusion of autophagosome with lysosome could be hindered by the intervention of Mtb, and thus it prevents the maturation of autophagosome and subsequently the autolysosomal degradation in macrophages. On the other hand, non-Mtb-containing autophagosomes continue to mature into autolysosomes [107]. Stimulation of autophagy by rapamycin, IFNγ, or vitamin D upregulated the autophagic flux, which in turn potentiated the clearance of Mtb through autophagy [106, 108].

Vitamin D-mediated inhibition of Mtb growth is dependent not only on the induction of autophagy' but also in autophagosomal maturation [108]. A ubiquitin-dependent mechanism has been demonstrated as a clearance mechanism of Mtb through autophagy [109, 110].

Autophagy exerts a dual role in tuberculosis. In addition to antibacterial activity, autophagy has been associated with anti-inflammatory activities. Mtb infection in Atg5 null increased the pulmonary inflammation [111]. Furthermore, autophagy in monocytes protected against Mtb infection. Autophagy augmentation enhanced antimicrobial activities against Mtb [112, 113]. Myeloid cell-specific deficiency of Atg5 significantly induced Mtb infection and reduced the survival rate of Mtb-infected mice [114]. Atg5 is protective against Mtb infection by preventing polymorphonuclear cell (PMN)-mediated immunopathology [114]. UPR has also been associated with the induction of apoptosis in macrophages and alveolar epithelial cells infected with Mtb. Apoptosis is a host defense mechanism which prevents the spread of mycobacterial infection to the surrounding tissue and is initiated by mycobacteria. Mtb H37Ra potentiates apoptosis in macrophages [115, 116] and secretes the highly immunogenic secretory antigenic target (ESAT-6), which perturbs the Ca^{2+} signaling. Disruption of Ca^{2+} homeostasis causes accumulation of ROS inside the cells, which in turn result in ER stress-induced apoptosis [117].

Mtb H37Rv has also been shown to suppress apoptosis. Phosphorylation of eIF2α affects the survival of Mtb and suppresses the eIF2α phosphorylation in a time-dependent manner [118]. Moreover, the modulation of the macrophage polarization is another mechanism employed by Mtb to circumvent the host immune system during tuberculous granulomas formation [119]. M1 macrophages induce inflammatory responses, promote granuloma formation and show resistance against intracellular parasites. The M2 macrophages mediate tissue repair and suppress the inflammatory process [119, 120]. M1 polarized macrophages demonstrate a higher ER stress to oppose Mtb. Infection with the Mtb H37Rv shows dominant expression of M2 macrophages, whereas the Mtb H37Ra infection resulted to increased levels of M1 macrophages [121].

Induction of apoptosis is more abundant in granulomas as compared to healthy tissue [122]. In addition, selective autophagy reduces the ER stress - induced apoptosis in Mtb-infected macrophages, and renders protection against Mtb. The BCL2 associated athanogene 2 (BAG2) induces reticulophagy, and suppresses ER stress. XBP1, transcriptionally inhibits BAG2 expression by binding to the *Bag2* promoter region. BAG2 activates autophagy through phosphorylation of BCL2 by MAPK during Mtb infection, and exerts anti-apoptotic effect. Indeed, BAG2 is a link between ER stress and autophagy in Mtb infection [123].

Autophagy and UPR in cystic fibrosis.

Cystic fibrosis (CF) is a lethal genetic disorder caused by mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, resulting in abnormal *CFTR* function. The most common mutation in *CFTR* gene is the deletion of phenyl alanine residue from the position-508 (F508del-*CFTR*) [124, 125]. The mutations in the *CFTR* gene impair protein synthesis; including gene transcription, protein translation, folding and trafficking [126]. Dysfunction of the *CFTR* protein causes severe disturbance to several key cellular mechanisms; including anion (chloride ion) transport, mucociliary-clearance, airway surface liquid (ASL) homeostasis [127], oxidative signaling [128], and autophagy.

Abnormal anion transport increases the mucin polymer cross-links and leads to thick mucus formation in the lungs [129]. This abnormal viscous mucus is an important characteristic of cystic fibrosis (CF) that blocks the airways. Those events result in chronic inflammation. Recurrent pulmonary infection occurs due to *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Burkholderia cepacia* [130].

Defective *CFTR* causes a maladaptive epithelial stress response associated with increased generation of reactive oxygen species (ROS), which in turn oxidizes and activates tissue transglutaminase (TG2) [131]. Activated TG2 targets several substrates, including Beclin 1 (BECN1). This is a major pro-autophagic protein which act as an activator of the phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3). Transamidation of BECN1 by TG2 causes the inhibition of autophagy, and accumulation of p62, which in turn regulate aggresome formation [132].

This disruption of the autophagic clearance causes the augmentation of inflammatory responses in *CFTR*-mutant mice [133]. Restoration of BECN1 by either beclin 1

overexpression or antioxidants, reverts the CF airway phenotype in vitro as well as in Scnn1b-transgenic and Cftr (F508del) homozygous mice [134]. Moreover, induction of autophagy by rapamycin or depletion of p62 decreased the bacterial survival and ameliorated the corresponding inflammatory responses [135].

CF is characterized by increased level of airway inflammation and chronic airway infection. Mutated CFTR in the pulmonary epithelial cells contributes to UPR activation [136]. Oxidative stress [137] and pathogens [138] may also accelerate CF through UPR induction. CFTR mutation can activate NF-kB directly [139]; as well as through UPR-mediated NF-kB activation [140]. Moreover, UPR induction is associated with the expansion of the ER Ca^{2+} store, which enhances inflammation [141].

Other studies have reported that ER stress suppresses the secretion of the neutrophil chemokines IL-8 and C-X-C motif chemokine ligand 1 (CXCL1); by inhibiting the activation of signal transducer and activator of transcription 3 (STAT3). Indeed, ER stress reduces neutrophil migration in the epithelial cells. Thus, ER stress alleviates the neutrophilic damage in CF airways, and reduces chemokine production [142].

Autophagy, UPR, acute lung injury and acute respiratory distress syndrome.

Acute lung injury (ALI) is an inflammatory lung disease, and it is characterized by the disruption of lung vascular endothelial and alveolar epithelial barriers [143, 144]. A severe form of acute lung injury is the acute respiratory distress syndrome (ARDS) [15, 145]. Different direct or indirect insults induce pulmonary inflammation, damage the alveolar-capillary membrane, and impair the function of the lungs [146, 147]. Those inflammatory processes lead to the accumulation of the protein-rich edema fluid in alveoli and interstitial space, reduction of alveolar fluid clearance, and impairment of the surfactant production and function [148]. Autophagy is involved in the pathogenesis of ALI/ ARDS.

Since the gas exchange capacity of the lung is significantly reduced during ARDS, mechanical ventilation (MV) becomes the most critical intervention in ARDS treatment [82]. It decreases the respiratory load of the patients by improving gas exchange. Even though MV supports ARDS patients, it has also been associated with aggravating lung injury [15, 149]. Interleukin (IL)-1β is one of the most biologically active proinflammatory cytokines which induces ALI [150]. Mice that do not express Atg16L1 (*Atg16l1* $^{fl/fl}$ *LysM* ^{Cre}), developed ALI. On the other hand, induction of autophagy protected the mice from LPS plus mechanical ventilation-induced ALI. It has also improved the arterial oxygenation and lung permeability, as well as it suppressed IL-1β production [151].

ALI may also occur due to high oxygen therapy (hyperoxia), a critical care setting to support tissue oxygenation. This is due to the enhanced ROS production in the lung capillary endothelial cells [152] and pulmonary epithelium [153]. Hyperoxia causes increased expression of autophagic markers; including autophagosome formation and pulmonary LC3B-II accumulation [154]. Suppression of LC3B by genetic interference promotes hyperoxia-induced cell death in pulmonary epithelial cells. Overexpression of LC3B showed

cellular protection after hyperoxia [154]. These results suggested the cytoprotective role of autophagic protein in hyperoxia-induced lung cell injury.

One of the most prominent pathophysiological characteristics of ALI is the lung microvascular endothelial barrier disruption. The endothelial cell-to-cell adherens junctions (AJs) are largely composed of vascular endothelial cadherin (VE-cadherin)/CDH5 and controls endothelial permeability [155]. Phosphorylation of CDH5 induces the dimer disassembly of CDH5 [156], which in turn increases endothelial permeability. The inflammatory tyrosine kinase Src [157] phosphorylates the tyrosine residues of CDH5 [158]. LC3 was associated with Src-selective autophagy [159].

Small GTPases mediate lung endothelial barrier function [160], including the RAB26. It was shown *in vivo* that RAB26 is downregulated in LPS-induced acute lung injury. On the contrary, LC3-II was associated with Src and promoted the degradation of the phosphorylated Src, when RAB26 was overexpressed. RAB26 induced autophagy by binding to ATG16L1 [161].

Autophagy exerted a protective role against cecal ligation and puncture (CLP)-induced acute lung injury. Elevated levels of LC3-II expression and accumulation of autophagosomes were detected in lung tissue of CLP-induced ALI [162]. Mice deficient in the Beclin 1 (*Becn1*$^{+/-}$) were more susceptible to CLP-induced ALI as compared to the wild-type mice. Moreover, *Becn1*$^{+/-}$ mice showed a significant reduction of bacterial clearance from blood and vital organs after CLP treatment. These results showed that Beclin-1 is involved in the survival of the CLP-treated mice by enhancing bacterial clearance [162]. Another study showed that the expression of Atg7, Beclin-1, and LC3B-II were upregulated in CLP-induced ALI [163].

Upregulation of autophagy is the underlying mechanism of the protective effects of FK866 in ALI. This is a noncompetitive nicotinamide phosphoribosyl transferase inhibitor. FK866 reduced lung injury score, edema, vascular leakage, and inflammatory infiltration. The inhibition of autophagy by 3-MA (3-methyladenine) reduced the beneficial effect of FK866 towards ALI [163]. It has also been reported that 3-MA markedly reduced the lung vascular damage, tissue edema, levels of proinflammatory mediators, and lung neutrophil sequestration in an aerosolized bacterial LPS inhalation mouse model of ALI [164]. Another study has also reported deleterious effect of autophagy in endothelial barrier disruption due to thrombin-induced ALI. Silencing of Beclin-1 in human pulmonary arterial endothelial cells caused the reduction of several proinflammatory genes and NF-kB activity. Moreover, knockdown of Beclin-1 reduced the thrombin-induced phosphorylation of RelA/p65, which is critical for the transcriptional activity of RelA/p65. Suppression of Beclin-1 prevented the disassembly of VE-cadherin, and thus protected against thrombin-induced EC barrier disruption [165].

UPR modulates vascular barrier function. It was reported that Kifunensine, a UPR suppressor, increases endothelial cell permeability by inducing the activation myosin light chain 2 (MLC2) and inhibiting the phosphorylation of cofilin [166]. Moreover, the heat shock protein 90 (Hsp90) inhibitors [167] and growth hormone releasing hormone (GHRH) antagonists [168] protect against the lipopolysaccharide (LPS)-induced ALI model. Hsp90

inhibitors have been shown to induce UPR and reduce the endothelial hyperpermeability [34, 169]. Those observations suggest the protective role of UPR in ALI/ARDS [37].

Conclusions

In our opinion, a carefully devised manipulation of autophagy in the context of UPR may deliver new therapeutic opportunities towards the inflammatory lung disease, including ALI/ARDS. Figure 1 concludes the information of our review.

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- The unfolded protein response mediates autophagy and vice versa
- Autophagy is involved in lung health and disease
- The unfolded protein response maintains lung homeostasis
- A novel therapeutic approach towards lung inflammatory disease may be developed based on autophagy manipulation
- Endoplasmic reticulum stress triggers the unfolded protein response activation

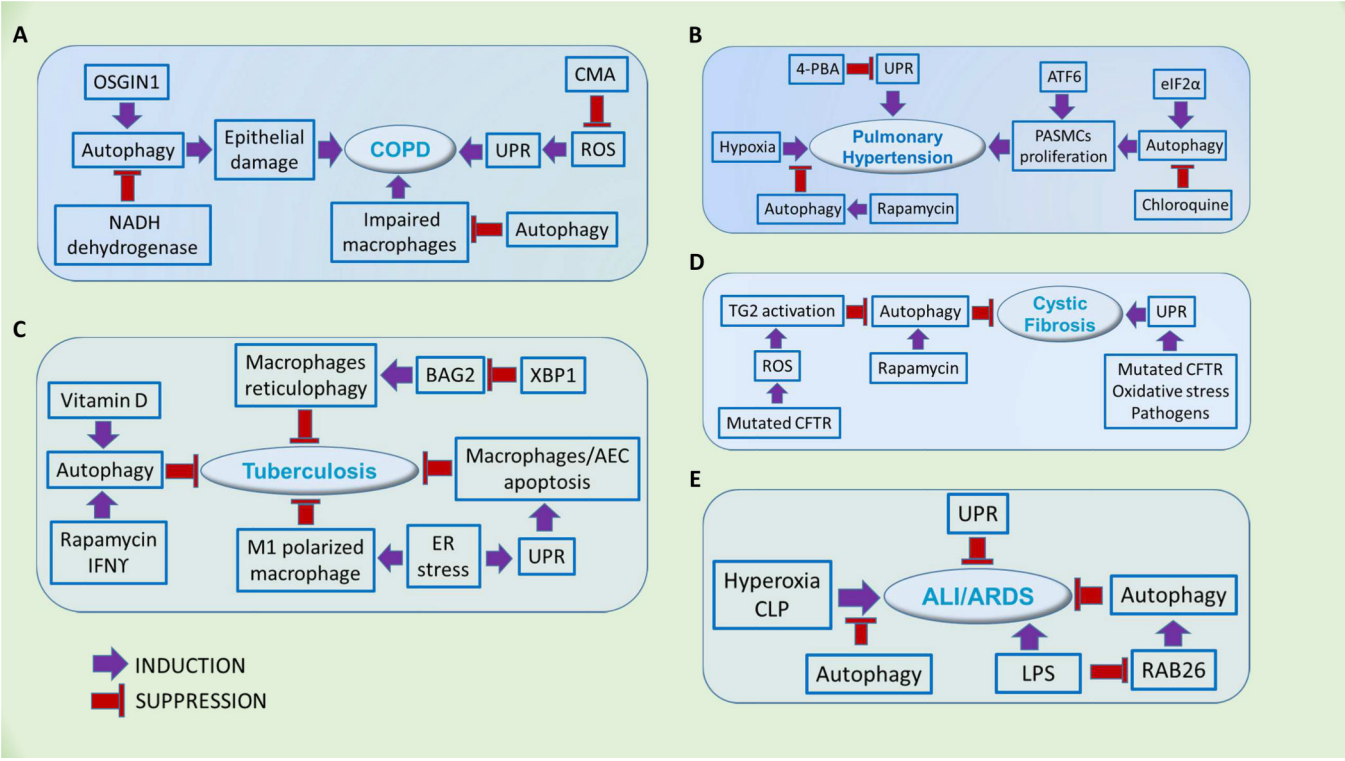

Figure 1: Effects of autophagy and UPR on the pathogenesis of lung disease.

(A) Chronic obstructive pulmonary disease (COPD): Increased levels of autophagy damages the airway epithelium which leads to COPD. OSGIN1 induces the expression of autophagy genes, whereas NADH dehydrogenase suppresses autophagy. However, autophagy protects the alveolar macrophages, and reduces persistent inflammation in COPD. Moreover, reactive oxygen species (ROS) induces UPR, which in turn contributes in COPD pathogenesis. Chaperone-mediated autophagy (CMA) suppresses the production of ROS, and hence, protects against COPD. **(B) Pulmonary hypertension (PH):** Autophagy protects against hypoxia-induced pulmonary hypertension. On the other hand, it accelerates in pulmonary artery smooth muscle cells (PASMCs) proliferation, inducing PH. Suppression of autophagy by chloroquine reduces PASMCs proliferation, and prevents the development of PH and vascular remodeling. UPR is associated with the PH pathogenesis, and suppression of UPR by 4-phenylbutyric acid (4-PBA) attenuates the PH. **(C) Tuberculosis:** Rapamycin, IFNγ and vitamin D inhibit tuberculosis by inducing autophagy. BCL2-associated athanogene 2 (BAG2) induces reticulophagy in *Mycobacterium tuberculosis* (Mtb)-infected macrophages, and protects against tuberculosis. Moreover, UPR promotes apoptosis in macrophages and alveolar epithelial cells (AEC) to suppress tuberculosis. M1 polarized macrophages exhibit higher ER stress, thus resisting against Mtb infection. **(D) Cystic fibrosis:** UPR induces cystic fibrosis, whereas autophagy defends against this disease. Mutated CFTR enhances ROS production, which suppresses autophagy by activating transglutaminase 2 (TG2). Moreover, Oxidative stress, mutated CFTR and pathogens can cause cystic fibrosis through UPR activation. **(E) Acute lung injury/ Acute**

respiratory distress syndrome (ALI/ARDS): Both autophagy and UPR exert protection against ALI/ARDS. RAB26 suppresses LPS-induced ALI by inducing autophagy.