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REVIEW

Research Progress on Autophagy Regulation by Active Ingredients of Traditional Chinese Medicine in the Treatment of Acute Lung Injury

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Abstract: Autophagy is a highly conserved process that maintains cell stability in eukaryotes, participates in the turnover of intracellular substances to maintain cell function, helps to resist pathogen invasion, and improves cell tolerance to environmental changes. Autophagy has been observed in many diseases, and the symptoms of these diseases are significantly improved by regulating autophagy. Autophagy is also involved in the development of lung diseases. Studies have shown that autophagy may play a beneficial or harmful role in acute lung injury (ALI), and ALI has been treated with traditional Chinese medicine designed to promote or inhibit autophagy. In this paper, the molecular mechanism and common pathways regulating autophagy and the relationship between autophagy and ALI are introduced, and the active ingredients of traditional Chinese medicine that improve ALI symptoms by regulating autophagy are summarized.

Keywords: autophagy, inflammation, oxidative stress, apoptosis

Introduction

Autophagy is a process in eukaryotes that protects cells and recycles metabolites by degrading misfolded proteins, processing damaged organelles such as mitochondria, the endoplasmic reticulum and peroxisomes, and removing invading pathogens, which can be used as raw materials for anabolic metabolism to maintain cell function in the absence of energy.[1] Autophagy avoids cell death and helps cells survive under stress conditions. In other contexts, autophagy also leads to cell death, which is known as autophagic cell death (ACD), an alternative death pathway that differs from apoptosis.[2] Acute lung injury (ALI) is a form of noncardiogenic pulmonary oedemaedema caused by pulmonary capillary endothelial and alveolar epithelial cell injury due to various internal and external factors, and it is a clinical syndrome characterized by progressive hypoxemia and dyspnoea.[3] Acute respiratory distress syndrome (ARDS) is a serious form of ALI with high morbidity and mortality rates that is common in the intensive care unit, and a drug that clearly improves the prognosis is unavailable.[4] Recent studies have shown that autophagy plays an important role in the treatment of neurological, tumour, cardiovascular, metabolic, infectious and autoimmune diseases.[5–7] The role of autophagy in lung diseases has also received increasing attention. However, because the mechanism of autophagy in lung diseases is still not fully understood and because of the limitations of drugs that specifically affect autophagy in the clinic, no significant progress in the treatment of ALI by regulating autophagy has been achieved.[6] Autophagy is regulated by multiple signalling pathways, and the synergistic effects of multiple targets may be regulated by traditional Chinese medicine. Many natural autophagy regulators have been identified in traditional Chinese medicine.[8] Here, we summarized the mechanism and regulatory pathway of autophagy, analysed the relationship between autophagy and acute lung injury, and summarized the current progress in the use of active components of traditional Chinese medicine to treat ALI models by regulating autophagy.

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Autophagy

Molecular Mechanism of Autophagy

Autophagy is a major regulator of innate and adaptive immunity, including the regulation of inflammation, antigen expression and bacterial clearance.[9] Autophagy is divided into microautophagy, macroautophagy and chaperone-mediated autophagy (CMA). Microautophagy is the direct engulfment of cytoplasmic contents through the deformation of the lysosomal membrane.[10] CMA is the process in which the target protein containing the KFERQ consensus motif is recognized and denatured by heat shock 70 protein 8 (HSPA8/HSC70), passes through the lysosomal membrane by binding to lysosome-associated membrane protein-2a (LAMP-2a) and is finally degraded in the lumen.[11] Macroautophagy is the process in which autophagosomes formed by de novo double-membrane vesicles composed of lipids from the plasma membrane, endoplasmic reticulum or Golgi engulf part of the cytoplasm and organelles. Then, autophagosomes degrade their contents by fusing with lysosomes and recycling the degraded byproducts, which are finally exported to the cytoplasm for reuse in macromolecule construction and metabolism.[12] Here, we mainly discuss the molecular mechanism of macroautophagy.

Autophagy is a highly regulated process that targets the elimination of aged or damaged organelles and participates in whole-cell processes in a nonselective manner.[13,14] Macroautophagy involves several key steps (Figure 1).

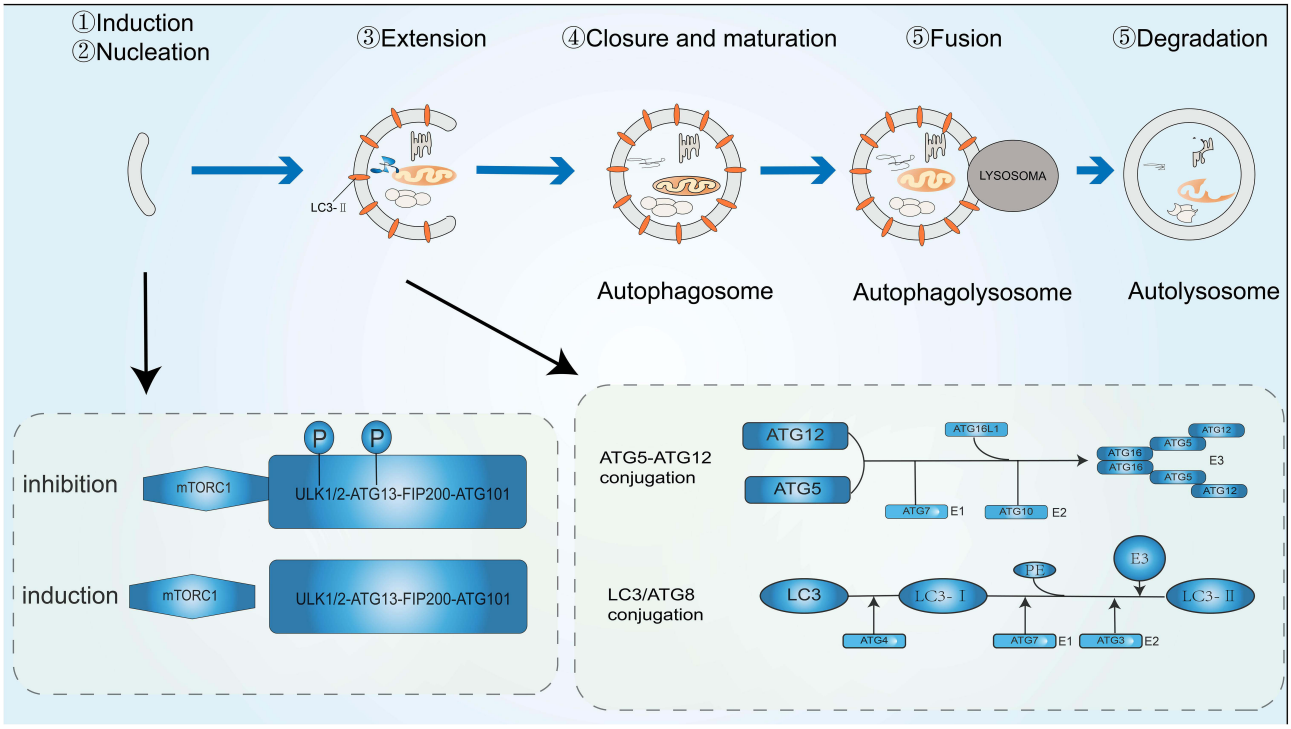

Figure 1 Molecular mechanism of autophagy. ① Induction: mTORC1 binds to the ULK1/2-induced complex (ULK1/2-ATG13-FIP200-ATG101) and inhibits its activation under nutrient-rich conditions. Under nutrient-deficient conditions, mTORC1 dissociates from ULK1/2-induced complexes, and the dephosphorylation of ATG13 and ULK1/2 induces autophagy.[130,131] ② Nucleation: Class III phosphatidylinositol 3-kinase (PIK3C3/VPS34) responds to the induction signal and forms the VPS34 complex by binding to ATG14, the human autophagy-related protein BECN1 (beclin-1, a mammalian ATG6 regulator) and myritic acid kinase (P150), which promote the production of phosphatidylinositol triphosphate (PI3P) specific to autophagosomes to participate in autophagy.[132,133] ③ Extension: Two ubiquitin-like binding systems are involved. The first system is the ATG5-ATG12 complex: the ATG12-ATG5-ATG16 complex is formed through the interaction of ATG12-ATG5 with ATG16L with the help of the ubiquitin Activase (E1)-like enzyme ATG7 and the ubiquitin binding enzyme (E2)-like enzyme ATG10, which is involved in phagocytic mass elongation.[134] The second system is the microtubule-associated protein 1 light chain 3 LC3 conjugate (LC3 is one of the mammalian homologues of ATG8). Processed LC3-II is produced by the continuous binding of phosphatidyl ethanolamine (PE) to LC3 in complex with protease ATG4, E1-like enzyme ATG7, E2-like enzyme ATG3, and ubiquitin ligase (E3)-like enzyme ATG12-ATG5-ATG16.[135] In addition, ATG9 has a role in recruiting lipids to expanded phagocytic masses, which is necessary for phagosome expansion.[136] LC3-II is recruited and integrated into the growing phagocytic mass via the ATG12-ATG5-ATG16 complex. ④ Closure and maturation: Phagocytic masses capture random or selected targets to form autophagosomes, and LC3-II is subsequently delipidated by ATG4 to release LC3. ⑤ Fusion and degradation: autolysosomes are formed by the fusion of autophagosomes and lysosomes. The inner membrane and luminal contents of autophagic vacuoles are degraded by lysosomal proteases, and this process is related to core protein complexes (SNAREs) and the small G proteins Rab7, LAMP-1 and LAMP-2.[137–139]

Pathways That Regulate Autophagy

Several signalling pathways have been reported to affect autophagy, and the central factor is mTORC1, which may mediate its effect on autophagy by inhibiting the ULK1/2 inducer complex during the autophagosome induction phase.[1] The main pathways that regulate macroautophagy are described below[5,15,16] (Figure 2). ① AMP-activated protein kinase (AMPK) is activated through the activation of adenosine 5-monophosphate. This enzyme induces autophagy through direct inhibition of mTORC1, activation of tuberous sclerosis complex 1/2 (TSC1/2) and phosphorylation of the ULK1/2 inducible complex.[17] ② PKB/AKT is activated by the activation of class I phosphatidylinositol 3-kinase (PI3K). This enzyme increases mTORC1 activity by inhibiting TSC1/2 and increasing Rheb (a small H-Ras-like GTPase) activity, thereby inhibiting autophagy.[18] ③ Autophagy is inhibited by the classical mitogen-activated protein kinase (MAPK) pathway (Ras-Raf-1-MEK1/2-ERK1/2). Extracellular regulated protein kinases 1/2 (ERK1/2) phosphorylate TSC2 and subsequently destroy the TSC1/2 complex, leading to mammalian target of rapamycin (mTOR) activation.[19] ④ The tumour suppressor gene P53 exerts a dual regulatory effect on autophagy. Cytosolic P53 inhibits autophagy by interfering with ULK1 complex activation. p53 enters the nucleus and increases its expression in response to DNA damage. P53 promotes autophagy by activating the AMPK pathway and increasing the transcription of DNA damage-regulated autophagy modulator 1 (DRAM1).[20] ⑤ C-Jun amino-terminal protein kinase 1 (JNK-1) is released from beclin-1 by phosphorylating antiapoptotic B-cell lymphoma 2 (BCL-2) or BCL-2-interacting mediator of cell death (BIM), which promotes the interaction between beclin-1 and VPS34 to induce autophagy.[21–23] ⑥ Death-related protein kinase (DAPK) also stimulates autophagy by phosphorylating beclin-1 and separating it from BCL-2. DAPK activates VPS34 by phosphorylating protein kinase D (PKD).[24] ⑦ KIAA0226/Rubicon inhibits autophagy by binding to UVRAG and negatively regulating beclin-1.[21,25] AMBRA1 promotes autophagy by directly binding to beclin-1, and SH3GLB1/HIF-1 induces autophagy by positively regulating beclin-1 through an interaction with UVRAG.[26] In addition, Liu et al found that a lipoquality A4 receptor agonist (BML-111) promoted autophagy to reduce lipopolysaccharide (LPS)-induced apoptosis and attenuate lung injury through

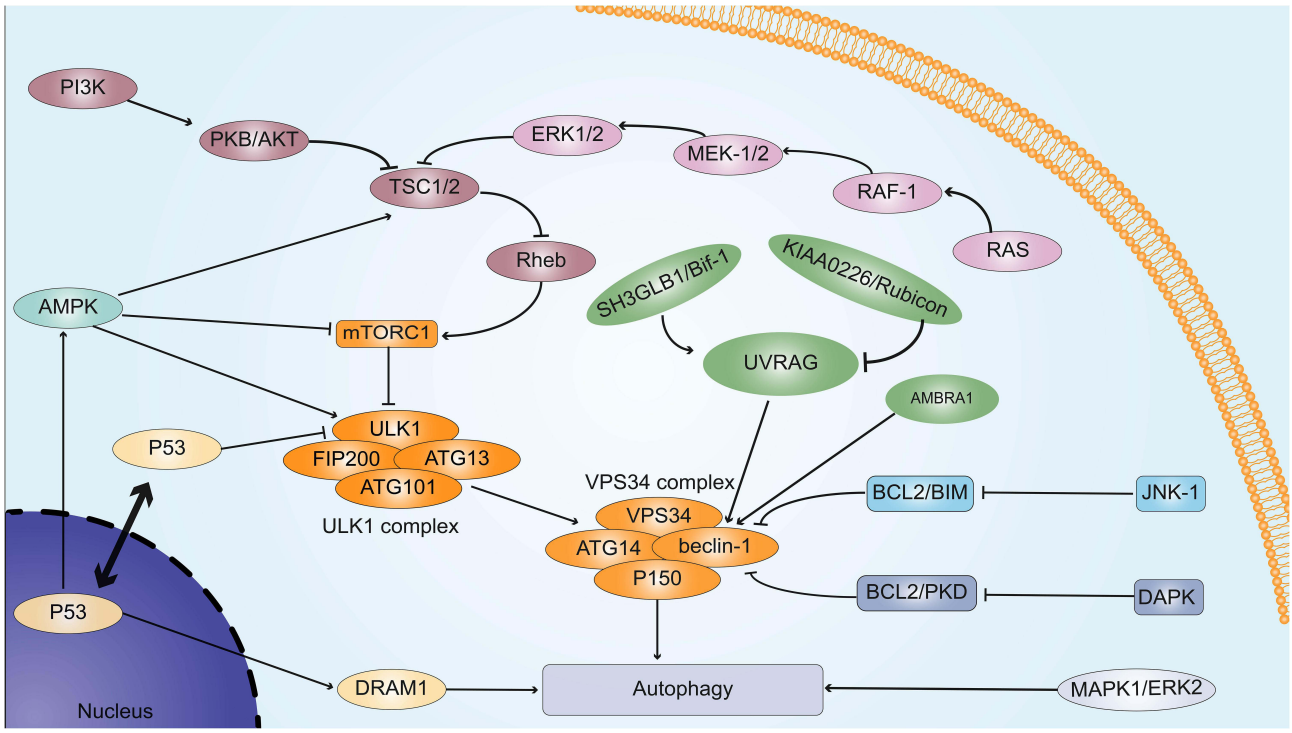

Figure 2 Pathways that regulate autophagy. →represents activation of the target, and ⊣represents inhibition of the target.
Abbreviations: AMPK, AMP-activated protein kinase; PI3K/Vps34, phosphatidyl inositol 3-kinase; PKB/Akt, protein kinase B; TSC, tuberous sclerosis complex; ERK1/2, extracellular regulated protein kinases 1/2; DRAM1, DNA damage-regulated autophagy modulator 1; mTOR, mammalian target of rapamycin; UVRAG, ultraviolet irradiation resistance-associated gene; SH3GLB1/Bif-1, bax-interacting factor 1; KIAA0226/Rubicon, RUN domain and Beclin1 interacting protein; AMBRA1, activating molecule in Beclin-1-regulated autophagy; JNK, c-Jun N-terminal kinase; BCL-2, b-cell lymphoma-2; BIM, BCL-2-interacting mediator of cell death; DAPK, death associated protein kinase; PKD, protein kinase D; MAPK, mitogen-activate d protein kinase.

the MAPK1/ERK2 signalling pathway rather than the mTOR pathway.[27] Based on these results, ERK2 activation also directly stimulates autophagy. These pathways regulate autophagy mainly by altering autophagosome formation and lysosome function and interfering with the fusion of autophagosomes and lysosomes, such as after treatment with bafilomycin A1 and chloroquine (CLQ).

Autophagy and ALI

ALI is a clinical syndrome characterized by diffuse alveolar injury caused by pulmonary infection, aspiration, pulmonary embolism, sepsis, pancreatitis, shock, trauma, blood transfusion and other factors. The common pathological manifestations are alveolar and interstitial oedema, alveolar haemorrhage, atelectasis, and hyaline membrane formation.[28] ALI pathogenesis involves inflammatory reactions, oxidative stress, apoptosis, endothelial injury, abnormal lung water clearance, and abnormal coagulation function.[29,30]

Autophagy and Inflammation

ALI is characterized by an uncontrolled inflammatory response in the lungs. Inflammation is the body's defence mechanism to resist the invasion of pathogens, while the anti-inflammatory response is conducive to reducing injury caused by excessive inflammatory reactions. Balancing proinflammatory and anti-inflammatory reactions is critical for the treatment of ALI.[31,32] Autophagy has been reported to regulate immune signalling cascades and balance immune responses.[33] Autophagy defends against pathogen invasion through the activation of many atypical pathways, such as the direct elimination of pathogens by "heterologous autophagy"[34] and the neutralization of pathogens through LC3-associated phagocytosis (LAP) without inducing complex formation.[35] In addition to improving host resistance, studies have shown that autophagy reduces damage after sepsis, improves host tolerance, and reduces the adverse effects of inflammation.[36] Therefore, the ultimate goal of autophagy may be to remove the threat while minimizing the damage to the host to balance the immune response and avoid long-term chronic diseases.[33] The relationship between autophagy and inflammation is shown in Figure 3.

mTOR regulates the IκB kinase (IKK) complex in a PKB/AKT-dependent manner and then activates the nuclear factor kappa-B (NF-κB) pathway in nuclear factor-activated B cells.[37] The autophagy activator rapamycin (RAPA) alleviates LPS-induced ALI by enhancing autophagy, inhibiting the activity of NF-κB and the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome, and reducing the levels of inflammatory cytokines and chemokines, including interleukin (IL)-1β, IL-18, IL-6, TNF-α and monocyte chemotactic protein-1 (MCP-1).[38] In addition, autophagy reduces the CXCL16 expression level and inhibits inflammation, thereby alleviating LPS-induced ALI in mice.[39] IL-1β is an early cytokine secreted by macrophages that stimulates polymorphonuclear neutrophils (PMNs) to release a large number of inflammatory mediators, and IL-1β activation is controlled by inflammasomes. Abnormal activation of the inflammasome stimulates the excessive activation of caspase-1 and produces a large amount of IL-1β, which induces excessive inflammatory reactions and causes tissue damage. Autophagy inhibits multiple steps in inflammasome activation and improves the symptoms of related diseases. Under normal conditions, autophagy contributes to the routine secretion of IL-1β to aid in defence.[40,41] Kiichi Nakahira et al[42] showed that LPS disrupts mitochondrial homeostasis in macrophages and increases mitochondrial reactive oxygen species (ROS) production, subsequently leading to mitochondrial membrane permeability transition (MPT) and the release of mitochondrial DNA (mtDNA) into the cytosol. mtDNA activates caspase-1 to activate IL-1β and IL-18. However, the MPT and ectopic transfer of mtDNA to the cytosol are mediated by the NLRP3 inflammasome. Autophagy maintains mitochondrial homeostasis by inhibiting mtDNA release mediated by the NLRP3 inflammasome, thus inhibiting caspase-1 activation and IL-1β and IL-18 release. This study provides a mechanistic explanation for the effect of autophagy on the inflammatory response by regulating caspase-1. Brenda G. Byrne et al[43] showed that inflammasomes mediate caspase-1 activation during microbial infection to induce an inflammatory response and apoptosis and that inflammasomes activate autophagy to increase the threshold of death in infected macrophages, enhancing tolerance. In addition, Qinjie Liu et al[44] showed that the increase in circulating mtDNA levels during sepsis promoted the excessive activation of the interferon gene (STING), which triggered an inflammatory cytokine storm. Furthermore, the STING pathway interferes with lysosomal acidification in a TANK binding kinase 1 (TBK1)-dependent manner, leading to impaired autophagosome

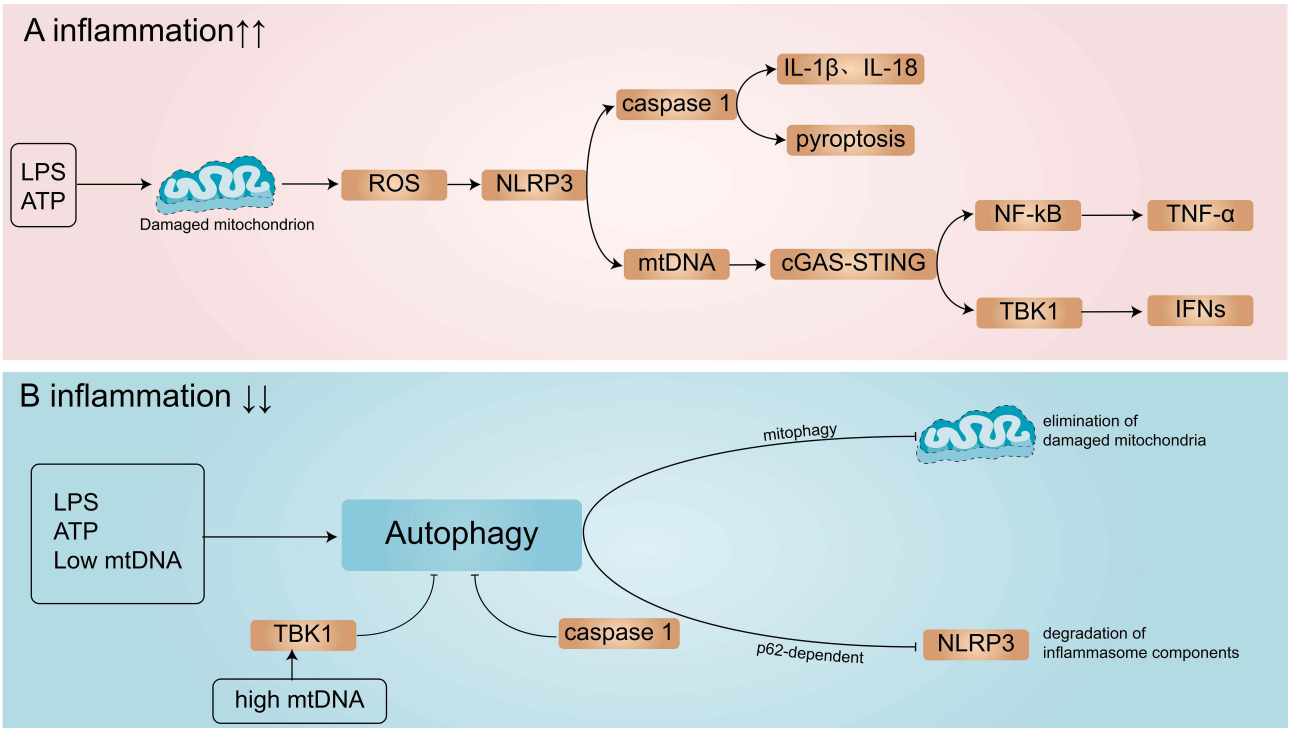

Figure 3 Autophagy and inflammation. (**A**) LPS and ATP stimulation impair mitochondrial homeostasis and lead to increased mitochondrial ROS production. Increased ROS production can activate NLRP3 inflammasome, which activates IL-1β and IL-18 by activating caspase-1, and can also mediate pyroptosis and cytoplasmic mtDNA release. Increased cytoplasmic mtDNA leads to activation of the cGAS-STING pathway, which in turn activates downstream TBK1 or NF-κB signaling pathways, leading to cytokine production and inflammatory responses. (**B**) LPS, ATP, and low-level mtDNA can induce autophagy, which can remove damaged mitochondria and degrade inflammasome components in a P62-dependent manner, thereby reducing the inflammatory response. A large amount of mtDNA leads to activation of the continuous STING pathway, which blocks autophagy flux in a TBK1-dependent manner, and caspase-1 also blocks autophagy by cutting autophagy-related proteins. →represents activation of the target, and ⊣represents inhibition of the target.

Abbreviations: LPS, lipopolysaccharide; ATP, adenosine triphosphate; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; NLRP3, nucleotide binding oligomerization domain-like receptor protein 3; cGAS, cyclic guanosine monophosphate-adenosine monophosphate synthase; STING, stimulator of interferon genes; NF-κB, nuclear factor kappa-B; TBK1, TANK binding kinase 1.

degradation and thereby causing sepsis-associated ALI (SALI). Knockout of the STING gene inhibits the increase in circulating mtDNA levels and increases the level of autophagy, which ameliorates inflammatory injury in SALI. The autophagy inducer RAPA decreases the levels of inflammatory factors and STING-related mRNA, while administration of the autophagy inhibitor 3-methyladenine (3-MA) exerts the opposite effect, indicating that increasing autophagy improved SALI. These studies confirmed that autophagy and inflammation affect each other, some inflammatory signals are inhibited by autophagy while inducing autophagy, and autophagy affects all parts of the inflammatory signalling cascade,[33] which provides a basis for regulating autophagy in the treatment of ALI.

Autophagy and Apoptosis

Autophagy determines the turnover of intracellular material, and apoptosis (type I cell death) determines cell turnover. Autophagy and apoptosis influence each other. Autophagy is an antiapoptotic process in most cases, and cell stress often induces autophagy first and activates apoptosis later, which is a self-protective mechanism.[24] Autophagy limits endogenous apoptosis caused by mitochondrial outer membrane permeabilization (MOMP) by removing damaged mitochondria.[45] Autophagy also selectively removes caspase-8, a key factor involved in exogenous apoptosis, to delay the occurrence of exogenous apoptosis.[46] In addition, autophagy selectively removes SRC tyrosine kinases to delay anoikis (anchorage-dependent cell death) induced by detachment from the extracellular matrix (ECM).[47] However, autophagosomes may also act as a platform for caspase-8 activation under specific circumstances.[48] In addition, due to the saturation of lysosome degradation, autophagosome accumulation promotes the opening of the mitochondrial permeability transition pore (mPTP), which leads to endogenous apoptosis caused by MOMP, promoting the release of

proapoptotic factors.[49] In addition, excessive autophagy leads to organelle depletion and induces ACD or type II cell death, which does not involve apoptotic effectors.[2] However, apoptosis leads to the cleavage of autophagy-related proteins through caspase activation, which inhibits autophagy and accelerates cell death.[50,51] The crosstalk between autophagy and apoptosis affects many pathophysiological processes. The relationship between autophagy and apoptosis is shown in Figure 4.

Autophagy and Lung Macrophage Apoptosis

Complement activation product (C5a) promotes alveolar macrophage (AM) activation and the release of proinflammatory cytokines and chemokines. Hu R et al[52] observed significantly increased autophagy in the AMs of ALI mice induced by intestinal IR and AMs cultured in vitro with C5a and exacerbated AM apoptosis, while the administration of C5a antibodies significantly inhibited autophagy and alleviated AM apoptosis. Mice lacking ATG5 (Mφ-ATG5-/- mice) did not form autophagosomes, the levels of inflammatory factors in bronchoalveolar lavage fluid (BALF) were decreased, lung injury was reduced, and AM apoptosis was significantly reduced; 3-MA exerted similar effects. Researchers have confirmed that autophagy induces AM apoptosis to promote ALI. In addition, this study also verified that autophagy induced by C5a was achieved through the degradation of the antiapoptotic protein BCL-2 via the binding of C5a to C5aR. The inhibition of autophagy attenuated AM apoptosis. M1 and M2 are two subtypes of AMs that play important roles in the development of ALI. M1 cells mainly play a defensive role, but the excessive secretion of proinflammatory factors causes lung injury. M2 cells mainly play roles in damage repair and immunosuppression, but the overexpression of M2 factors exacerbates fibrosis. M1 and M2 cells can be converted into each other, and balancing the levels of M1 and M2 cells is critical in the treatment of ALI.[53] In the AMs of ALI rats treated with LPS and rat NR8383 macrophages cultured in vitro, autophagy was enhanced, AM apoptosis was increased, M2-to-M1 polarization was increased, and lung tissue injury was exacerbated. Hydrogen-rich saline (HRS) treatment inhibited autophagy, reduced AM apoptosis, promoted the polarization of M1 macrophages to M2 macrophages, and ameliorated lung injury. The same effect was observed after the administration of the autophagy inhibitor 3-MA. AM apoptosis may be caused by the excessive accumulation of autophagosomes and the fusion and degradation of lysosomes. Autophagy inhibition reduced the excessive accumulation of autophagosomes and thus attenuated ALI.[54] Based on these studies, autophagy was involved in AM apoptosis during ALI, and the regulation of autophagy ameliorated ALI by reducing AM apoptosis and promoting M1-to-M2 polarization.

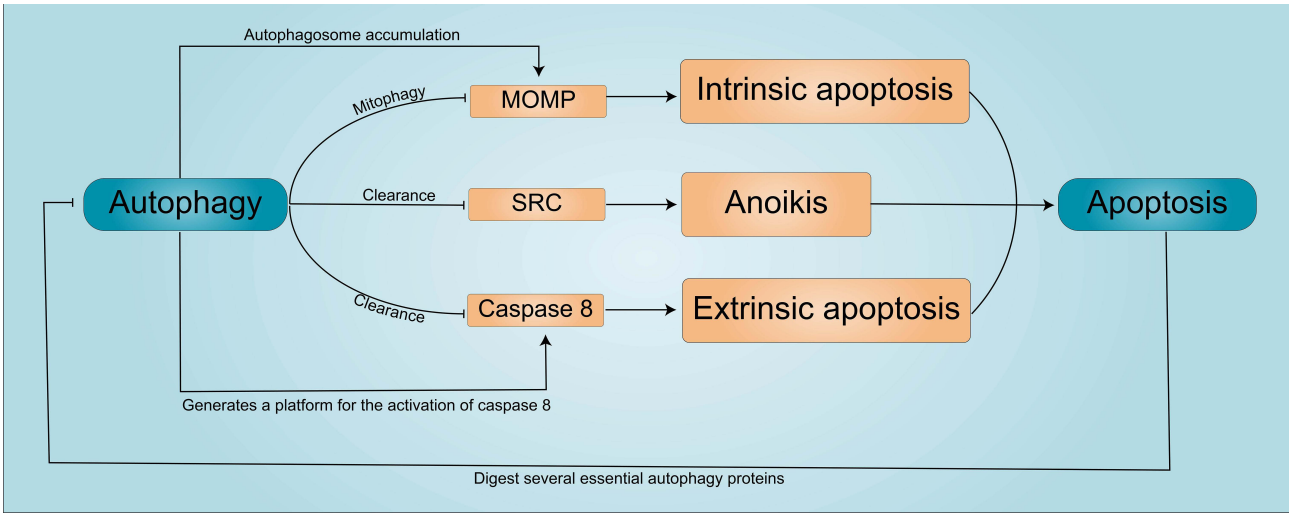

Figure 4 Autophagy and apoptosis. →represents activation of the target, and ⊣represents inhibition of the target.
Abbreviation: MOMP, mitochondrial outer membrane permeabilization.

Autophagy and Alveolar Type II Epithelial Cell Apoptosis

The proliferation and differentiation of type II alveolar epithelial cells (AECIIs) complement damaged and necrotic AECIs. AECIIs also secrete pulmonary surfactant (PS) to reduce alveolar surface tension and participate in immune defences and lung water clearance.[55] Therefore, AECIIs play an important role in the pathogenesis of ALI. Studies[56] have shown that cobalt II chloride ($CoCl_2$) treatment induces autophagy and apoptosis in rat alveolar type II epithelial RLE-6TN cells, and $CoCl_2$ stimulation causes severe damage to the endoplasmic reticulum and mitochondria in RLE-6TN cells and increases ROS generation. After the addition of the autophagy inhibitor 3-MA, RLE-6TN cell apoptosis was significantly increased, mitochondrial damage was further exacerbated, ROS generation was significantly increased, and the levels of cleaved caspase-9 and cleaved caspase-3 were significantly increased, indicating that autophagy inhibition may increase AECII apoptosis through the caspase-9 pathway. Other studies have shown that autophagy is critical for the formation of the osmilophilic lamellar body (LB) and the secretion of PS by AECIIs, and ATG7-deficient mice exhibit impaired LB formation,[57] suggesting that autophagy also plays an important role in maintaining surface tension in alveoli.

Autophagy and Pulmonary Vascular Endothelial Cell Apoptosis

According to a previous report[58] using a model of lung injury induced by ischaemia/reperfusion (I/R), pulmonary microvascular colorectal cancer cells (PMVECs) induce autophagy under oxygen glucose deprivation (OGD)/reoxygenation conditions. When HPMECs were cocultured with bone marrow-derived mesenchymal stem cells (BM-MSCs) in vitro, autophagy was further enhanced, endothelial permeability and the mitochondrial membrane potential ($\Delta\Psi m$) were significantly improved, and HPMVEC apoptosis was decreased. The authors also verified that the mechanism by which BM-MSCs enhance autophagy involved reducing the levels of class I PI3K and p-AKT.

Autophagy and Oxidative Stress

Under pathological conditions, oxidative stress and associated cellular components induce oxidative damage when ROS production exceeds the scavenging capacity of the antioxidant system.[59] ROS are mainly derived from the mitochondrial inner membrane respiratory chain and peroxisomes, and the physiological level of ROS plays an important role in maintaining cell function.[60] The overproduction of ROS under stress conditions activates autophagy, which negatively regulates ROS levels by removing damaged mitochondria and peroxisomes. The nuclear factor erythroid-derived 2-like 2 (Nrf2) pathway reduces oxidative stress-mediated damage, and Nrf2 is a key transcription factor in the antioxidant system that resists oxidative stress by interacting with antioxidant response elements (AREs) or electrophile response elements (EpREs) in vivo. Kelch-like ECH-associated protein 1 (Keap1) is a repressor of Nrf2.[61,62] p62/SQSTM1 is a ubiquitin-dependent autophagy degradation receptor that promotes the release of Nrf2 by binding to Keap1. Nrf2 expression increases the level of p62/SQSTM1, thus forming a positive feedback loop, and then the Keap1-P62 complex is recruited to autophagosomes for degradation.[63] The p62 positive feedback loop also increases mitophagy and reduces oxidative stress-induced cell damage.[64] The relationship between autophagy and oxidative stress is shown in Figure 5.

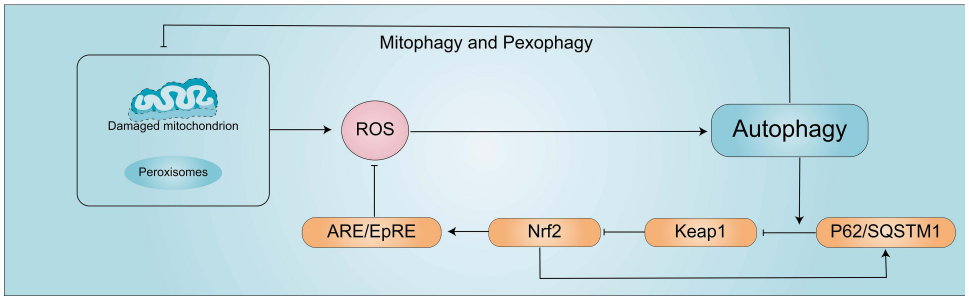

Figure 5 Autophagy and oxidative stress. →represents activation of the target, and ⊣represents inhibition of the target.
Abbreviations: ROS, reactive oxygen species; ARE, antioxidant response elements; EpRE, electrophile response elements; Nrf2, nuclear factor erythroid-derived 2-like 2; Keap1, Kelch-like ECH-associated protein 1; p62/SQSTM1, sequestosome 1.

Lung tissue injury in rats stimulated with sodium hydrogen sulfide (NaHS) was significantly exacerbated, and the levels of ROS, malondialdehyde (MDA) and myeloperoxidase (MPO) in the lungs were significantly increased. Based on these results, NaHS induces an oxidative stress response and PMN infiltration into the lungs of rats, leading to lung injury. After inhibiting autophagy with 3-MA, lung injury was further exacerbated, and oxidative stress and PMN infiltration were further enhanced, which proved that oxidative stress and inflammation were attenuated, and NaHS-induced ALI was ameliorated by autophagy.[65]

Autophagy and Endothelial Injury

Autophagy and Neutrophil Transendothelial Migration

PMNs that cross the endothelial cell (EC) barrier to inflammatory sites and eliminate pathogens are important components of the mechanisms by which the body prevents pathogen invasion. However, uncontrolled neutrophil recruitment may cause tissue damage.[66,67] The mass migration of PMNs in ALI destroys intercellular junctions, and these cells release a large number of toxic inflammatory mediators, leading to the apoptosis of alveolar epithelial cells, which is an important cause of pulmonary oedemaedema.[68]

Studies have shown that EC autophagy regulates the interaction between ECs and neutrophils under cellular stress conditions such as hypoxia, infection and trauma.[69] This result was further confirmed by a study by Natalia Reglero-Real et al.[70] The researchers found that autophagy at the EC junctions in microvessels and venules was enhanced in IR/LPS-stimulated wild-type mice, while autophagy at the EC junction was decreased in ATG5-deficient mice, and transendothelial cell migration (TEM) of PMNs was significantly increased. After the administration of an autophagy-inducing peptide (Tat-beclin-1/Tat-BECN1), autophagy was further increased in wild-type mice, and TEM was significantly inhibited, indicating that autophagy was involved in regulating TEM and tissue infiltration by modulating EC junctions. In addition, the adherence of PMNs to venous ECs in wild-type mice and ATG5-deficient mice was not significantly changed. However, the amount of TEM, the number of platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) pores, the opening duration, and the width and enrichment of PECAM-1 and VE-cadherin (VE-Cad) junctions were significantly increased in ATG5-deficient mice. The levels of intercellular cell adhesion molecule-1 (ICAM-1/CD54), ICAM-2, vascular cell adhesion molecule-1 (VCAM-1), E-selectin and other cell surface proteins were increased, but their mRNA expression levels were not different. This finding indicated that autophagy defects resulted in the accumulation of connexins on the surface of ECs rather than an increase in gene expression. This study further confirmed that ECs mediate the degradation of PECAM-1 and other cell surface molecules through the LAP-like noncanonical autophagy pathway, and EC autophagy exerted the same effects on monocyte and eosinophil transport. In conclusion, autophagy inhibits the TEM of various leukocytes by regulating the interjunction structure and molecular composition of ECs during acute inflammation, which provides a basis for regulating autophagy to reduce the tissue infiltration of inflammatory cells.

Changes in Autophagy and Endothelial Permeability

ALI is characterized by the accumulation of protein-rich fluid in the tissue space and alveoli, which is closely related to the destruction of the alveolar capillary barrier and an increase in endothelial permeability.[29] The adhesion activity and dimer decomposition of cadherin 5 (CDH5/VE-Cad) are critical for maintaining adherens junctions (AJs) between ECs and reducing vascular endothelial permeability. CDH5 phosphorylation mediated by the tyrosine kinase SRC dissociates dimers and increases endothelial permeability. LPS reduces the autophagy levels of HPMECs, while RAB26 is involved in autophagy induction through direct interactions with ATG16L1. By increasing autophagy, SRC is degraded, and CDH5 phosphorylation is reduced, thereby maintaining EC integrity.[71–74] However, another study[75] showed that LPS increased autophagy in mouse lungs, and autophagy inhibition reduced thrombin-induced VE-Cad cleavage to maintain AJ integrity. LPS induced autophagy, increased the levels of VCAM-1, MCP-1, IL-1β and myeloperoxidase (MPO) and exacerbated pulmonary vascular leakage and tissue oedemaedema. However, after the administration of the autophagy inhibitor 3-MA, these indexes were significantly decreased, VE-Cad expression was increased, pulmonary vascular leakage and tissue oedemaedema were improved, and thrombin-induced EC barrier disruption was also observed after siRNA-ATG5 transfection, which confirmed that autophagy impaired EC barrier function by increasing VE-Cad

cleavage. These results showed that LPS exerted opposite effects on autophagy. Studies have shown that LPS exerts different effects on autophagy in different lung cells. The former study measured the autophagy levels of HPMECs cultured in vitro, while the latter measured the autophagy levels of cells in the mouse lungs, which contain a variety of cells, and the two results may differ. The effects of autophagy on VE-Cad expression were also inconsistent between the two studies, suggesting that autophagy may regulate VE-Cad expression through various pathways to maintain the balance of endothelial permeability. Damage to the endothelial barrier is caused not only by the destruction of intercellular connections but also by EC apoptosis and changes in endothelial permeability caused by inflammatory factors, which results from comprehensive effects. The relationship between autophagy and endothelial injury is shown in Figure 6.

These studies showed that autophagy is involved in regulating multiple ALI processes, such as the inflammatory response, oxidative stress, apoptosis, and endothelial injury. Due to the crosstalk between the signalling molecules in these processes, autophagy is likely to improve ALI through a comprehensive effect mediated by multiple pathways. Although the specific mechanism by which autophagy affects these pathological processes in ALI is not fully understood and the interactions between various pathways require further study, these studies clarify that approaches designed to regulate autophagy can treat ALI.

Pathways That Regulate Autophagy to Treat ALI

PI3K/AKT/mTOR Pathway

Studies have shown that BM-MSCs decrease autophagy in RAW264.7 murine macrophages by activating the PI3K/AKT/mTOR signalling pathway under OGD conditions.[76] LPS stimulation enhances autophagy by inhibiting the PI3K/AKT/mTOR signalling pathway. Cystic fibrosis transmembrane conductance regulator (CFTR) and hydrogen sulfide (H_2S) have been shown to inhibit autophagy by activating the PI3K/AKT/mTOR signalling pathway, thereby alleviating the LPS-induced inflammatory response in ALI mice.[77,78]

C-Jun N-Terminal Kinase (JNK) Pathway

FK866 is a specific inhibitor of nicotinamide phosphoribosyl transferase (NAMPT).[79] NAMPT is a rate-limiting enzyme in the NAD salvage synthesis pathway. FK866 promotes the consumption of NAD and increases ATP levels.[80] Increased ATP levels inhibit the activation of AMPK and JNK. The p-JNK level was increased in the CLP-induced ALI mouse model, while FK866 decreased the p-JNK level and increased autophagy. The JNK inhibitor SP600125 also enhanced autophagy and attenuated lung injury, and similar findings were also obtained in vitro. Thus, FK866 enhances autophagy

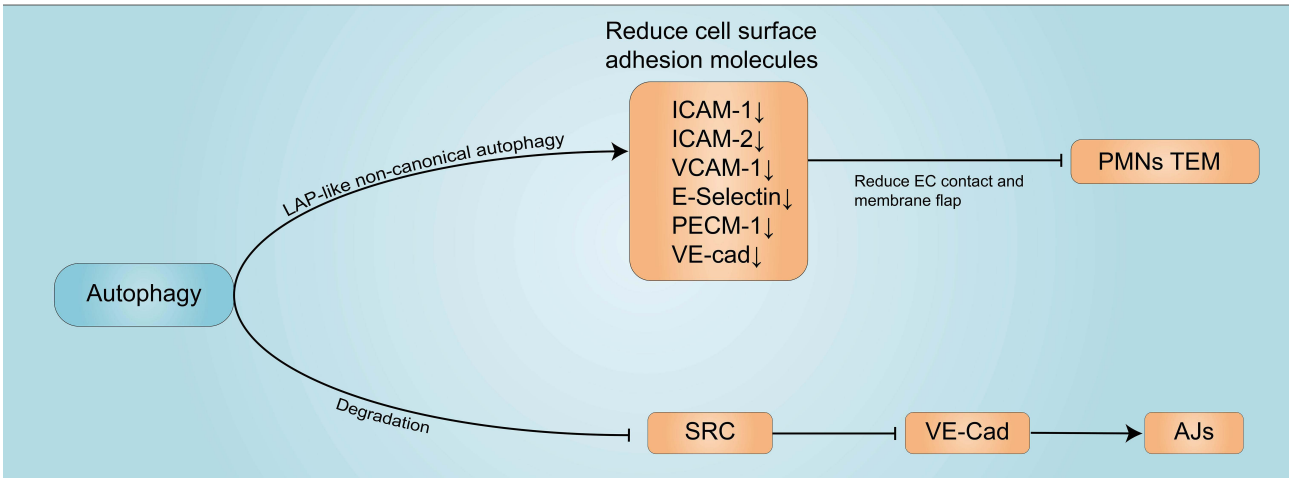

Figure 6 Autophagy and endothelial injury. →represents activation of the target, and ⊣represents inhibition of the target.
Abbreviations: ICAM-1, intercellular cell adhesion molecule-1; ICAM-2, intercellular cell adhesion molecule-2; VCAM-1, vascular cell adhesion molecule-1; PECAM-1, platelet endothelial cell adhesion molecule-1; VE-Cad, VE-cadherin; TEM, transendothelial cell migration; AJs, adherens junctions.

and improves CLP-induced ALI in mice by inhibiting JNK activity.[81] However, JNK has been shown to actively regulate autophagy in other diseases,[23,82,83] and researchers have proposed that the same signalling pathway may have different roles in different diseases.

ERK1/2/mTOR/Stat3 Pathway

ALI is one of the complications of traumatic brain injury (TBI). Studies have shown that autophagy activation by the ERK1/2/mTOR/Stat3 pathway attenuates the inflammatory response, oxidative stress and cell apoptosis induced by TBI in ALI mice.[84]

AMPK/mTOR Pathway

HRS prevents ALI through its antioxidant effect. Yong Wang et al reported that HRS reduces ROS production, inhibits autophagy induced by the AMPK/mTOR pathway and improves LPS-induced ALI in cell-based experiments and animal experiments.[85]

PTEN-Induced Putative Kinase 1 (PINK1)/Parkin Pathway

PINK1 degradation is inhibited when mitochondria are damaged, and PINK1 interacts with Parkin (a ubiquitin (Ub) E3 ligase encoded by the Park2 gene) to promote its phosphorylation in the cytoplasm and recruitment to mitochondria. This process in turn triggers the migration of Ub and the LC3-binding adaptor protein P62, which links damaged mitochondria to mitophagy and is ultimately degraded by fusing with lysosomes.[86,87] Bcl-2 inhibits mitophagy by inhibiting the recruitment of Parkin from the cytosol to mitochondria and alleviates LPS-induced injury in A549 lung epithelial cells and ALI mice.[88]

Active Ingredients of Traditional Chinese Medicine That Ameliorate ALI by Regulating Autophagy

We searched databases such as PubMed, Web of Science, and China National Knowledge Infrastructure (CNKI) to identify the main active ingredients of traditional Chinese medicine that improve ALI by regulating autophagy (Table 1), with priority given to articles published in the last 10 years. The terms used in the search were “Chinese medicine and autophagy and acute lung injury”, “Chinese herbal medicine and autophagy and acute lung injury”, and “traditional medicine and autophagy and acute lung injury”.

Glycyrrhizic Acid

Glycyrrhizae Radix et Rhizoma (Gancao) comes from the roots and rhizomes of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat. and *Glycyrrhiza glabra* L. and is the most commonly used ingredient in prescriptions. Glycyrrhizic acid (GA), 18-β-glycyrrhetic acid (18β-GA) and other triterpenoids, flavonoids and a small amount of polysaccharides are the main components of *Glycyrrhiza* roots. GA activates autophagy, reduces the release of inflammatory factors, and attenuates the symptoms of LPS-induced ALI in mice, but 3-MA reversed the protective effects of GA. In addition, p-PI3K, p-AKT and p-mTOR protein levels were significantly reduced after GA treatment. Therefore, the authors postulated that the activation of autophagy by GA may be partially achieved by inhibiting the PI3K-AKT-mTOR pathway.[89]

Astragaloside IV

Astragali Radix (Huangqi, also known as astragalus) is the dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge., whose main active component is astragaloside, as well as polysaccharides, flavonoids, choline, betaine and trace amounts of folic acid. LPS induced autophagy in MLE-12 cells, decreased cell viability and tight junctions, and increased cell apoptosis. After treatment with the autophagy inducer RAPA, cell viability and tight junctions were further inhibited, and these effects were reversed by 3-MA treatment. Thus, the induction of autophagy in the ARDS cell model in vitro was harmful. Astragaloside IV (AS-IV) inhibits oxidative stress and

Table 1 The Role of Traditional Chinese Medicine in ALI Treatment by Regulating Autophagy

Active Ingredients of Traditional Chinese Medicine	Sample	Effects on Autophagy	Pathways that Regulate Autophagy	Pharmacological Effects	Reference
Glycyrrhizic acid	RAW264.7 cells and male BALB/c mice	Activate	PI3K-AKT-mTOR	Reduces the secretion of inflammatory cytokines	[89]
Astragaloside IV	MLE-12 lung epithelial cells	Inhibit	None	Increases cell viability and tight junctions	[90]
Ginsenoside Rg1	MLE-12 lung epithelial cells	Activate	None	Autophagy activates Nrf2 signalling to inhibit NF-κB transcriptional activity and attenuates inflammation and apoptosis	[92]
Sinomenine	RAW264.7 cells and female ICR mice	Activate	None	Antioxidant and anti-inflammatory effects	[95]
Curcumin	Adult male SD rat	Activate	None	Reduces inflammation	[96]
Emodin	Male BALB/c mice	Activate	None	Reduces inflammation	[97]
Tetrahydropalmatine	Male Sprague–Dawley rats	Inhibit	PI3K-AKT-mTOR	Reduces inflammatory cell infiltration and alveolar wall oedema	[98]
Oxyberberine	A549 cells and male BALB/c mice	Inhibit	None	Reduces apoptosis, inflammation and ROS production	[102]
Oxymatrine	Male Sprague–Dawley rats	Inhibit	None	Reduces inflammation and oxidative stress	[104]
Hydroxytyrosol	Male BALB/C mice	Activate	SIRT/MAPK	Reduces inflammation	[106]
Resveratrol	Male C57/BL6 mice	Inhibit	PLSCR-3	Reduces mitochondrial dysfunction	[110]
Sophoridine	RAW264.7 cells and mice	Activate	TLR4/MYD88/NF-κB	Reduces inflammation	[112]
N-butanol extract of Jingfangsan	Male ICR mice	Activate	None	Reduces inflammation and oxidative stress	[115]
Hispolon	Male ICR mice	Inhibit	None	Reduces inflammation and oxidative stress	[117]
Cinobufagin	HBE cells and C57/BL6 mice	Promote	p53/mTOR	Reduce inflammation and inhibit epithelial cell apoptosis	[119]
Polydatin	Beas-2B cells and C57/BL6 mice	Promote	Parkin-dependent mitophagy	Against mitochondria-dependent apoptosis	[121]
Thalictrum minus L.	Male C57/BL6 mice	Inhibit	None	Reduce inflammation, oxidative stress and apoptosis	[123]
YiQiFuMai lyophilized injection	Male C57/BL6 mice	Inhibit	mTOR	Reduces inflammation and oxidative stress	[125]

inflammation, inhibits LPS-induced autophagy, and increases cell viability and tight junctions. Therefore, AS-IV may directly or indirectly inhibit the initiation of autophagy by inhibiting oxidative stress and inflammation.[90] However, this study did not verify the causal relationship between inhibition of the inflammatory response and autophagy inhibition.

Ginsenoside Rg1

Radix et Rhizoma Ginseng (Renshen) is the dried root of the *Araliaceae* plant *Panar ginseng* C. A. Mey. Modern pharmaceutical studies have shown that *P. ginseng* exerts powerful anti-inflammatory, antioxidant and antiapoptotic effects,[91] and its main active ingredient is ginsenoside, along with monosaccharides, polysaccharides, amino acids, vitamins and enzymes. Ginsenoside Rg1 activates autophagy in the LPS-induced ALI model established using MLE-12 mouse lung epithelial cells, and the increase in autophagy activates Nrf2 signalling to block the phosphorylation of P65 and reduce the level of cleaved caspase-3. The phosphorylation of p65 activates NF-κB, leading to inflammation and apoptosis. The levels of P-P65 and cleaved caspase-3 increased after the Nrf2 gene was silenced, indicating that the protective effect of ginsenoside Rg1 on ALI involved activating Nrf2 signalling through autophagy and inhibiting NF-κB signalling.[92] Ginsenoside Rg2 was also reported to attenuate LPS-induced ALI by inhibiting the TLR4-PI3K-AKT-mTOR signalling pathway and Raf-1-MEK-ERK pathway and enhancing the Keap1-Nrf2-HO1 signalling pathway. As a result, PMN infiltration and tissue oedemaedema were reduced, and the levels of proinflammatory factors were decreased in ALI mice.[93] Another study showed that ginsenoside Rg3 attenuated LPS-induced ALI by activating the C-MER proto-oncogene tyrosine kinase (MerTK)-dependent PI3K-AKT-mTOR pathway.[94] These two studies did not explain the relationship between the protective effects of ginsenosides Rg2 and Rg3 on ALI mice and autophagy, but these signalling pathways are important pathways regulating autophagy. Therefore, autophagy may be involved in the therapeutic effect of ginsenosides RG2/3 on ALI.

Sinomenine

Sinomenii caulis (Qingfengteng) is a common medicine in rheumatology, and the main active ingredient is sinomenine (SIN). Nrf2 directly regulates the activity of the haem oxygenase-1 (HO-1) promoter and activates NAD(P)H quinone oxidoreductase 1 (NQO1). Both HO-1 and NQO1 are important antioxidant enzymes, while Keap1 inhibits Nrf2 activity. Studies have shown that SIN increases the expression of Nrf2, HO-1 and NQO1, decreases Keap1 expression, decreases the release of the inflammatory cytokines IL-6, TNF-α and IL-1β, decreases the levels of MDA and increases the level of superoxide dismutase (SOD) in vivo and in vitro. Thus, LPS-induced ALI was alleviated. Treatment with the Nrf2 inhibitor brusatol (BRU) reversed the anti-inflammatory and antioxidant effects of SIN, thus verifying that SIN exerts anti-inflammatory and antioxidant effects by activating Nrf2 signalling. Furthermore, this study also verified the connection between Nrf2 signalling and autophagy. SIN pretreatment significantly increased the expression of LC3II/I, Atg5 and beclin-1 in vitro and in vivo, and the expression of LC3II/I, Atg5 and beclin-1 was inhibited after treatment with the Nrf2 inhibitor BRU, suggesting that SIN activated the Nrf2 pathway. Nrf2 activation is related to the activation of the downstream signalling pathway in autophagy.[95] However, this study did not further verify the effect of autophagy regulation on the anti-inflammatory and antioxidant effects of SIN.

Curcumin

Curcumae longae Rhizoma (Jianghuang) is the dried rhizome of the *turmeric* plant *Curcuma longa* L., which mainly contains curcumin, gingerolene and other essential oils, as well as small amounts of sugars and trace elements. In rats with LPS-induced ALI, the expression levels of the autophagy-related genes ATG5 and ATG7 and the autophagy marker proteins LC-3 and beclin-1 were increased in the curcumin treatment group, and inflammatory cell infiltration in lung tissue and the levels of the inflammatory factors TNF-α, IL-1β and IL-6 in BALF were decreased. However, the 3-MA group showed the opposite results, which proved that curcumin reduced lung inflammation in ALI mice by enhancing autophagy.[96]

Emodin

Rhei Radix et Rhizoma (Dahuang) is the rhizome of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf. and *Rheum officinale* Baill. of *Polygonaceae*, which tastes bitter and cold, enters the stomach, large intestine and liver meridian and has the effects of clearing heat from the gut, cooling and detoxifying the blood, and channelling the meridian by dretting. In emodin-treated mice with LPS-induced ALI, the expression of autophagy-related proteins in lung tissue was significantly increased, the infiltration of inflammatory cells in lung tissue and the expression of the inflammatory factors TNF-α and IL-6 were decreased, pulmonary vascular permeability was alleviated, and pulmonary oedemaedema was improved. However, the administration of 3-MA reversed the protective effect of emodin on ALI mice, indicating that emodin alleviated ALI induced by endotoxemia by increasing autophagy.[97] The study did not verify the pathway through which emodin regulates autophagy.

Tetrahydropalmatine

Tetrahydropalmatine (THP) is the active ingredient of Corydalis Rhizoma (Yanhusuo), the dried tuber of *Corydalis yanhusuo* W. T. Wang. In the rat model of ALI induced by limb I/R, the expression of beclin-1 and LC3II in lung tissue decreased, the expression of LC3I and P62 increased, and the phosphorylation of PI3K/AKT/mTOR was significantly inhibited, indicating that limb I/R activated autophagy by inhibiting the PI3K/AKT/mTOR pathway. However, THP reversed these changes, alleviated inflammatory cell infiltration and lung tissue oedemaedema and alleviated limb I/R-induced ALI, and 3-MA treatment had the same effect. However, RAPA combined with THP treatment significantly inhibited the protective effect of THP on ALI, which proved that THP prevented limb I/R-induced ALI by inhibiting autophagy through the activation of the PI3K/AKT/mTOR pathway.[98] Levo-tetrahydropalmatine (L-THP), an enantiomer of tetrahydropalmatine, is a nonnarcotic analgesic with sedative, calming and hypnotic effects. L-THP was reported to activate the AMPK-mTOR-ULK1 and ROS-JNK-ATG7 cascades and induce ERK/AKT signalling to promote autophagy,[99] which suggested additional pathways by which tetrahydropalmatine regulates autophagy.

Oxyberberine

Berberine is a quaternary ammonium salt isoquinoline alkaloid isolated from the Coptidis rhizome (Huanglian) that has antibacterial, anti-inflammatory and antioxidant effects.[100] Oxyberberine has a stronger anti-inflammatory effect and is safe.[101] In mice with LPS-induced ALI and lung epithelial A549 cells, oxyberberine treatment significantly improved pulmonary oedemaedema, neutrophil infiltration and inflammatory factor expression, inhibited apoptosis and ROS production in A549 cells, and inhibited mitophagy induced by LPS in A549 cells. Moreover, after Baf was used to inhibit mitophagy, the protective effect of oxyberberine completely disappeared, indicating that oxyberberine improved ALI by inhibiting excess mitophagy.[102]

Oxymatrine

Oxymatrine (OMT) is a quinoline alkaloid isolated from Radix Sophorae Flavescentis (Kushen) that has anti-inflammatory, antiapoptotic, antifibrotic and ameliorative effects on myocardial injury.[103] Compared with nondiabetic rats, diabetic rats were more likely to experience ALI and myocardial injury after myocardial I/R. Myocardial I/R-induced lung autophagy was activated in diabetic rats with ALI. After treatment with the autophagy inducer RAPA, the levels of inflammatory factors such as TNF-α, IL-6 and IL-8 in the BALF of rats with myocardial I/R-induced ALI were further increased, SOD levels were decreased, and the 15-F2t-isoprostane (15-F2t-isoprostane) level was increased. The autophagy inhibitor 3-MA reversed these changes, while OMT inhibited autophagy in a dose-dependent manner, suggesting that OMT ameliorated myocardial I/R-induced ALI in diabetic rats by inhibiting autophagy.[104]

Hydroxytyrosol

Hydroxytyrosol (HT) is a natural polyphenolic compound derived from *Canarium album* (Lour.) Rauesch. with anti-inflammatory, antioxidant and antibacterial activities[105] that exert protective effects on cardiovascular, neurological and metabolic diseases, and its efficacy in lung diseases is receiving increasing attention. HT significantly reduces the levels of proinflammatory factors and chemokines in the BALF of mice with LPS-induced ALI and improves lung hyperaemia, oedema and leukocyte accumulation in ALI mice. Moreover, HT treatment promotes autophagy, decreases the levels of phosphorylated MAPK proteins (ERK, JNK and p38) and increases the expression of SIRT (sirtuins) in the lungs of ALI mice. Therefore, HT promotes autophagy to improve ALI in mice through the SIRT/MAPK pathway.[106] However, this experiment did not verify the relationship between the promotion of autophagy and inflammation or the relationship between the P-MAPK and SIRT protein levels and the level of autophagy.

Resveratrol

Resveratrol (RSV) is an important active component in *Polygoni Cuspidati Rhizoma et Radix, Mori Fructus* and *Rhei Radix et Rhizoma* with anti-inflammatory, antioxidant, cardioprotective and senescence prevention effects.[107] Phospholipid scramblase 3 (PLSCR-3) promotes the transfer of cardiolipin (CL) to the outer mitochondrial membrane, and CL induces mitophagy by directly interacting with LC3 through its specific binding site.[108,109] RSV reduces mitochondrial damage, inhibits mitophagy and attenuates ALI in mice with CLP-induced sepsis. In addition, PLSCL-3 overexpression eliminates RSV-mediated inhibition of mitophagy and reverses the improvements in mitochondrial dysfunction and the protective effects of RSV on CLP-induced ALI. Therefore, RSV may inhibit mitophagy by inhibiting PLSCL-3 activation, thereby alleviating mitochondrial dysfunction and ALI.[110]

Sophoridine

Sophoridine is a tetracyclic quinolisidine alkaloid derived from *Sophora alopecuroides* L., *Euchresta japonica* Benth. and *Sophora moocrorftinan* with antitumour, anti-inflammatory and antiviral activities.[111] Studies have shown[112] that mice with LPS-induced ALI exhibit hyperaemia in the large alveolar wall, thickening of the lung interstitium, inflammatory cell infiltration, and increased expression of inflammatory factors (HMGB1, TNF-α, IL-6 and IL-1β) in lung tissue and serum. The expression of autophagy-related proteins was increased, autophagy flux was increased, and TLR4, MYD88, P65 and P-mTOR protein levels in lung tissues were also increased. Increased levels of inflammatory factors were also observed in LPS-stimulated RAW264.7 cell culture medium, and these cells underwent autophagy. Levels of the TLR4, MYD88, P65 and P-mTOR proteins and TLR4 and MYD88 mRNA expression in lung tissue were decreased after sophoridine treatment. Furthermore, the autophagy level was increased, and LPS-induced ALI was attenuated. Therefore, sophoridine is presumed to promote autophagy and improve ALI through the TLR4/MYD88/NF-κB and mTOR pathways. However, this study did not verify a link between autophagy activation and improvements in ALI symptoms, nor did it confirm a causal relationship between the TLR4/MYD88/NF-κB pathway and autophagy enhancement.

N-Butanol Extract of Jingfangsan

Jingfangsan is composed of a 1:1 combination of Schizonepetae Herba (Jingjie) and Saposhnikoviae Radix (Fangfeng), whose active ingredient in an N-butanol extract exerts anti-inflammatory and anti-allergic effects.[113,114] JFNE-A is a bioactive ingredient fractionated from this compound. According to a previous study,[115] JFNE-A treatment significantly alleviates LPS-induced pathological changes in the lungs of ALI mice. The levels of IL-6, TNF-α, IL-1β, M-CSF and IFN-γ in BALF were decreased, and the level of phosphorylated NF-κB (p65) was decreased. Based on these results, JFNE-A reduces the expression of inflammatory factors by inhibiting the NF-κB signalling pathway. In addition, JFNE-A enhanced the antioxidant effects on ALI mice and reduced lung injury by increasing Nrf2, HO-1 and SOD1 protein levels. JFNE-A also significantly promotes autophagy in the lung tissue of ALI mice. Thus, JFNE-A treats ALI by regulating autophagy, oxidative stress, and the NF-κB signalling pathway, and autophagy reduces ROS release by

scavenging damaged mitochondria, which in turn inhibits inflammation and oxidative stress. However, this study did not provide further evidence of a link.

Hispolon

Hispolon is a natural polyphenolic compound that is the main active ingredient of *Sanghuangporus sanghuang*. It has the effects of inhibiting cancer cell metastasis and the cell cycle, inducing cancer cell apoptosis, anti-inflammation, anti-virus and antioxidation.[116] Studies have shown that hispolon can improve the pathological changes in lung tissue in LPS-induced ALI mice, reduce pulmonary oedema, and reduce inflammatory cell infiltration and the release of inflammatory mediators. The above effects are related to the inhibition of IKK/IκBα/NF-κB, MAPK, PI3K/Akt/mTOR, LKB1/CaMKK-AMPK signalling pathways to reduce inflammation, enhance Keap1/Nrf2/HO-1 axis and peroxisome proliferator-activated receptor γ (PPARγ) activity to inhibit oxidative stress, reduce the expression of apoptosis-related proteins such as Bax, Bcl-2 and caspase-3, and reduce ER stress and inhibit autophagy.[117] However, this study only verified the anti-inflammatory, antioxidant, apoptosis inhibition, ER stress reduction and autophagy inhibition effects of hispolon and did not further verify the crosstalk between various effects or the relationship between autophagy inhibition and the above signalling pathways.

Cinobufagin

Cinobufagin is one of the components of toad venom and plays an important role in the treatment of various cancers.[118] Cinobufagin reduced LPS-induced alveolar structural damage, pulmonary oedema and inflammatory cell infiltration in mouse lung tissue; reduced the protein concentration, cell number and levels of inflammatory factors such as IL-1β, IL-6 and TNF-α in BALF; increased p-p53 levels; and decreased p-mTOR levels, which could be reversed by 3-MA. In vitro experiments also confirmed that cinobufagin can inhibit LPS-induced apoptosis of human bronchial epithelial cells (HBE cells), reduce the production of IL-1β, IL-6 and TNF-α, and activate the p53/mTOR pathway to induce autophagy.[119] In addition, the p53 inhibitor pifithrin-μ was used to confirm the protective effect of cinobufagin and the relationship between autophagy induced by cinobufagin and the p53/mTOR pathway.[119]

Polydatin

Polydatin is an extract of Polygoni Cuspidati Rhizoma et Radix (Huzhang), which has strong anti-inflammatory, antioxidation and antiapoptotic effects. It is beneficial in the treatment of cardiovascular, respiratory, digestive, neurological, rheumatic and other diseases.[120] It has been reported that polydatin can improve LPS-induced ALI. Li Tao[121] et al found that polydatin can activate mitophagy and reduce mitochondrial-dependent apoptosis to improve ALI in vivo and in vitro by establishing an ARDS model. The mitophagy inhibitor mdivi-1 reversed the above protective effect and further confirmed that polydatin activated the Parkin-dependent mitophagy pathway by siRNA silencing of the Parkin gene.

Thalictrum Minus L

Thalictrum minus L. (TML) is a folk medicine used to treat pulmonary infection, dysentery and fungal infection in China and Mongolia. It has been reported that TML can treat ALI induced by LPS or particulate matter (PM) in mice.[122,123] Its effect on improving LPS-induced ALI in mice was confirmed to be related to autophagy.[123] Studies have shown that LPS significantly upregulated the expression of the autophagy-related protein LC3-II, and the expression of LC3-II decreased after TML treatment.[123] However, the increase or decrease in LC3-II alone does not represent the change in autophagy level, and this study did not confirm the relationship between the change in autophagy level and the improvement in ALI and did not further verify the pathway regulating autophagy.

YiQiFuMai Lyophilized Injection

YiQiFuMai (YQFM) lyophilized injection is a traditional Chinese medicine compound injection commonly used in the clinic. Its main components include 65 compounds,[124] which are commonly used in the treatment of heart disease, cerebrovascular disease and sepsis. Studies have shown that YQFM can inhibit the infiltration of inflammatory cells in

the lungs of PM-induced ALI mice, reduce the number of total cells and proteins in BALF, inhibit the production of NO, TNF-α, and IL-1β, reduce the production of PM-induced autophagic vacuoles, reduce the upregulation of TLR4 and MyD88 caused by PM, enhance mTOR phosphorylation, and reduce the expression of autophagy-related proteins LC3-II and Beclin1, indicating that the anti-inflammatory effect of YQFM is related to the inhibition of TLR4/MyD88 signalling and reduction of autophagy.[125] The TLR4/MyD88 pathway can induce the production of inflammatory mediators and oxidative factors such as TNF-α and IL-1β, which may indirectly activate autophagy, but this study did not confirm the direct regulatory relationship between TLR4 and autophagy.

Discussion

With the development of molecular biology and genetic engineering techniques, autophagy has been shown to usually exert a protective effect rather than a harmful effect, except in some diseases in which abnormal autophagy activation may induce or exacerbate the disease. Autophagy is often the final attempt of cells to respond to survival challenges and threats, although this process is often accompanied by cell death.[7,126] However, many previous studies only examined autophagy levels by counting autophagosomes or measuring LC3-II levels. In this case, the exact opposite process might occur. Autophagy is increased when too many autophagosomes form, when lysosomal activity is impaired or when autophagosomes do not effectively fuse with lysosomes for degradation. Therefore, more accurate methods must be developed to measure autophagy levels to allow the results to provide a scientific and reliable basis for clinical practice. Autophagic flux reflects the entire process of autophagosome formation to degradation, which is more representative of autophagy levels. At present, the main methods used to monitor autophagy flux include measurements of LC3 turnover, the mRFP-GFP-LC3 dual fluorescence autophagy indicator system, and measurements of the total levels of autophagy substrates (such as GFP-LC3 or p62).[127]

Traditional Chinese medicine has great potential in the treatment of ALI. Numerous studies have shown that the active ingredients of traditional Chinese medicines exert beneficial effects on all aspects of the pathogenesis of ALI.[128] However, research related to the treatment of ALI by regulating autophagy with traditional Chinese medicine is still incomplete. First, although ALI is caused by different aetiologies and different stages of the onset of ALI have been identified, autophagy develops in different directions. Autophagy may be insufficient, or excessive autophagy or accumulation of autophagosomes may occur.[129] The active ingredients of traditional Chinese medicines regulate changes in ALI and ameliorate its symptoms. However, the specific stage of autophagy affected by the active ingredients of traditional Chinese medicines to improve ALI has not been clearly elucidated. For example, researchers have not determined whether these active ingredients promote or inhibit the process of autophagosome formation or interfere with or enhance the fusion and degradation of autophagosomes and lysosomes. Second, the specific mechanism by which the active ingredients of traditional Chinese medicines improve ALI by regulating autophagy requires further examination. For example, the specific pathway by which autophagy is regulated to inhibit the ALI-associated inflammatory response, the mechanism by which autophagy regulation reduces apoptosis in ALI epithelial cells and its effect on EC permeability, and the mechanism by which autophagy regulation alleviates the oxidative stress response are still not fully understood. Finally, as many active ingredients of traditional Chinese medicines affect autophagy, they may also affect biological processes through other pathways independent of autophagy. These pharmacological effects may not necessarily be mediated by autophagy, and many atypical autophagy pathways have been identified. Therefore, the design of autophagy-related studies should be more rigorous. Further elucidation of the mechanisms by which traditional Chinese medicines affect autophagy may provide new choices for drugs to treat ALI.

ALI is a highly complex and dynamic pathological process involving a wide range of areas. Multiple signalling pathways are involved, which exhibit crosstalk with each other. Therefore, a single treatment approach will clearly be ineffective. The regulation of autophagy plays a role in ALI through various mechanisms, including anti-inflammatory, antioxidant, and antiapoptotic effects, reducing endothelial damage, regulating immunity and other aspects. In addition, TCM treatment is a comprehensive diagnostic and treatment model guided by the holistic concept that integrates fuzheng, dispels evil, adjusts Yin and Yang, gives priority to prevention, and seeks the essence of treatment. Traditional Chinese medicine is expected to play a more important role in the treatment of ALI by regulating autophagy.

Traditional Chinese medicine prescriptions are the treasures of traditional Chinese medicine. At present, we have explained the multitargeted therapeutic mechanism of some classical patent medicines or prescriptions from the perspective of modern medicine through scientific research, which corresponds to their traditional efficacy. This aspect could be a future direction of polypill research. After understanding the mechanism of action of each active component of traditional Chinese medicines, compound preparation is a feasible approach to treat ALI. However, the compositions of traditional Chinese medicines are complicated, and the composition of compound preparations is complicated because of the interaction between various drug components. TCM focuses on syndrome differentiation and treatment and applies proper therapeutic measures consistent with the season, local conditions and individual characteristics, and the prescription also requires following the compatibility law of the monarch, minister, and others. These characteristics are not only a unique and powerful advantage of traditional Chinese medicine but also a major challenge for traditional Chinese medicine research.

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Disclosure

The authors report no conflicts of interest related to this work.

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