European Review for Medical and Pharmacological Sciences 2019; 23: 1674-1680

Autophagy in pulmonary macrophages mediates lung inflammatory injury via c-Src tyrosine kinase pathway activation during mechanical ventilation

G. LI[1], Y. LI[2], S.-F. ZHENG[1], Y.-B. HAN[1], Q.-L. BAI[3], T. ZHAO[1]

[1]Department of Anesthesiology, People's Hospital of Rizhao, Jining Medical University, Rizhao, China
[2]Department of Anesthesiology, The Third People's Hospital of Qingdao, Qingdao, China
[3]Operation Room, People's Hospital of Zhangqiu District, Jinan, China

Abstract. – OBJECTIVE: It has been clearly demonstrated that autophagy plays a critical role in mechanical ventilation-Induced lung injury (VILI). Herein, we first evaluated the mutual effects of autophagy and c-Src signaling on the lung inflammatory response to mechanical ventilation.

MATERIALS AND METHODS: Mice were respectively subjected to a lower or higher lung stretch induced by mechanical ventilation with low (7 mL/kg) or high (28 mL/kg) tidal volume, before measuring the activation of autophagy and c-Src signaling through LC3 lipidation and c-Src phosphorylation, respectively. Bone marrow-derived macrophages (BMDMs) were transfected with Atg5 siRNA and administered to AM-depleted mice to generate an autophagy-deficient phenotype, and c-Src signaling was evaluated by Western blot assay to determine the impact of autophagy on c-Src activation during VILI. Afterwards, the c-Src pathway was then blocked using PP2, prior to the evaluation of polymorphonuclear neutrophils (PMN), total cell counts in BAL fluid, and lung injury scores, in order to elucidate the role of the c-Src pathway in autophagy-mediated VILI.

RESULTS: Both LC3-II and p-c-Src were remarkably increased after mechanical ventilation, in a time-dependent and tidal volume-dependent manner. Moreover, c-Src phosphorylation induced by ventilation was significantly compromised in autophagy-deficient mice. On the other hand, LC3-II expression did not change due to c-Src signaling abolishment. But the inflammatory response induced by injurious ventilation was markedly attenuated by PP2 or AM-abolishment, shown by PMN and total cell counts in BAL fluid, as well as lung injury scores.

CONCLUSIONS: Our results suggested that autophagy caused VILI *via* regulating c-Src activation, which implies that c-Src may serve as a promising therapeutic target in VILI.

Key Words
Ventilator-induced lung injury (VILI); Autophagy; Macrophage; c-Src; Inflammation.

Introduction

Mechanical ventilation is extensively applied in the clinically intensive care of patients with acute respiratory failure and anesthesia. Albeit its benefits on gas exchange, this supportive therapy may cause lung stretch and thereby initiate a pro-inflammatory response and lung lesions termed as ventilation-induced lung injury (VILI)[1]. VILI is characterized by impaired oxygenation capacity, an influx of inflammatory cells, increased alveolar membrane permeability, and destruction of alveolar epithelial cell junctions, which may, in turn, lead to secondary pulmonary dysfunction and acute respiratory distress syndrome (ARDS)[2,3] and ultimately contribute to mortality in patients subjected to mechanical ventilation[4]. In response to passive stretch, pro-inflammatory cytokines are generated and released by a variety of cell types, including alveolar epithelium[5], endothelium[4], neutrophils[6], and in particular, macrophages[7,8]. The released cytokines are involved in a systemic inflammatory response and the triggering or/and exacerbation of VILI[9].

Autophagy ("self-eating") is a mechanism of catabolic processing of unnecessary or dysfunctional cellular components within a specialized double-membrane vesicle, referred to as an autophagosome, which later fuses with the lysosome[10]. In this way, it serves to improve cellular energy generation under nutrient limitation conditions

Corresponding Author: Tao Zhao, MM; e-mail: 347754658@qq.com

and crucially acts in cell survival and homeostasis[11]. Upon initiation of autophagy, the cytosolic LC3-I isoform is derived from LC3, a mammalian homolog of the yeast Atg8, as a result of cleavage by Atg4B protein. LC3-I is subsequently conjugated to phosphatidylethanolamine to generate the lapidated is of LC3-II, which is further recruited as a constitutive element on the autophagosomal inner membrane[12]. In addition to nutrient deprivation, autophagy can also be induced by other irritant metabolic stresses such as hypoxia and pathogen infection[13,14].

Mizushima[15] has elaborated the role that autophagy plays, other than starvation adaptation, in a variety of physiological and pathophysiological processes, including elimination of intracellular protein and organelle, anti-tumourigenesis, development, and antigen presentation. Intriguingly, in some situations, cytosolic replication intermediates are recognized by TLR7, a crucial Toll-like receptors (TLRs) family member that typically recognizes viral snRNA and initiates immune responses, following transportation to endosomes/lysosomes through autophagy pathway, giving rise to secretion of inflammatory cytokines[16]. Beyond that, autophagy participates in the regulation of interleukin-1β (IL-1β) production, a pro-inflammatory cytokine considered as a commendable hallmark of ventilator-triggered lung inflammation[17]. Actually, autophagy or chest rates diverse signaling pathways that are involved in innate immunity and lung inflammation in response to mechanical ventilation[18,19]. Herein, available evidence has been extended by our observations on the autophagy specifically activated in lung macrophages demonstrating that the autophagy activation initiates mechanical ventilation-triggered inflammatory responses through the activation of a proto-oncogene tyrosine-protein kinase Src (also refers to as c-Src). Thus, c-Src tyrosine kinase pathway can be deemed as a novel therapeutic target that effectively inhibits the autophagy-mediated lung inflammatory responses and thereby conceivably alleviates VILI during mechanical ventilation.

Materials and Methods

Animals

C57BL/6J male mice (weighing 25-30 g) were obtained from the Laboratory Animal Center of Shandong University (Jinan, Shandong, China). Animals were housed in a specific pathogen-free condition, fed with autoclaved food, and used in experiments at 8-12 week of age. Mice were handled under a protocol approved by the Laboratory Animal Ethics Committee of People's Hospital of Rizhao (Rizhao, Shandong, China).

Experimental Procedures

A well-established VILI mouse model was described in our previous study[18]. In brief, mice were anesthetized with an intraperitoneal injection of a pentobarbital sodium/ketamine mixture (75 mg/kg) and infusion of pentobarbital (15 mg/kg every 30 min) for maintenance. Mice were randomly subjected to mechanical ventilation with a low tidal volume of 7 mL/kg (a respiratory rate of 120 breath/min) or a high tidal volume of 28 mL/kg (a respiratory rate of 60 times/min). The ventilation parameters were set as follows: an I/E ratio of 1:2 and a fraction of inspired oxygen of 21%. All experiments were conducted with room air and a positive end-expiratory pressure of 0 cmH_2O. In some experiments, mice were pre-treated with c-Src inhibitor (1 μg/kg PP2) for 30 min prior to anesthesia. After exposure to mechanical ventilation, mice were euthanized, and lung injury was evaluated by scoring, bronchoalveolar lavage (BAL) fluid measurement, and histological analysis of lung tissues.

AM Depletion in Mice

Phosphatidylserine, phosphatidylcholine, and cholesterol at a molar ratio of 1:6:4 were mixed in chloroform to generate clodronate liposome solution, before the removal of chloroform by rotary evaporation at 40°C. The clodronate liposome was then filtered through a 200-nm filter and then delivered to anesthetized mice *via* nebulization.

Preparation of Mouse BMDMs and Atg5 Knockdown

Bone marrow-derived macrophages (BMDMs) were isolated from femurs of C57/BL6J mice. Bone marrow was flushed out from femurs using ~5 mL PBS. The released cell suspensions were centrifuged at 500 g for 5 min, and the pellets were resuspended in macrophage complete medium [DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (fetal bovine serum), 20% L-929 cells conditioned medium, 10 mM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin] (Gibco, Rockville, MD, USA). Afterwards, cells were collected into a sterile plastic dish containing 10 ml macrophage complete medium and maintained at 37°C and 5% CO_2. The medium was renewed 72 h later. The adherent cells were harvested when they were ~95% pure mac-

rophages, as indicated by the macrophage surface marker F40/8 (Santa Cruz, Santa Cruz, CA, USA).

The concentration of BMDMs was adjusted to 2×10^6 cells/well and transfected with Atg5 siRNA or a scrambled siRNA (GenePharma, Shanghai, China) according to the manufacturer's protocol. All experiments were conducted 48 h after transfection. The transfected BMDMs were administered to AM-depleted mice through jugular venous cannula 30 min prior to ventilation. An equal volume of saline was also administered as negative control.

PMN and Total Cell Counts

BAL fluid was collected by lavage for three times, using an intratracheal injection of 1 mL of phosphate-buffered saline (PBS) and subsequent gentle aspiration, followed by centrifugation at 400 g for 5 min and resuspending pellets in PBS. 200-μL resuspended cells (1×105 cells/ml) were cytospun onto slides with a cytocentrifuge and slides were then stained with a Diff-Quick dye (Dade Behring, Newark, DE, USA). Cells were examined and counted under a light microscopy. The total cell count was manually determined with a hemocytometer, and the percentage of PMNs was assessed after counting at least 300 cells in random fields.

Immunoblotting

Mouse tissue fragments were lysed in radioimmunoprecipitation assay (RIPA) buffer supplemented with a protease inhibitor cocktail (Roche, Basel, Switzerland). Proteins were then separated on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, (Billerica, MA, USA), and subsequently probed with appropriate antibodies. Signals were detected by chemiluminescence using enhanced chemiluminescence (ECL) system (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

One-way ANOVA and Student's *t*-tests or Tukey were used to assess significant differences from at least three independent experiments. They were followed by a Post-Hoc test. Statistical analysis was conducted using the Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA). Data are presented as means ± SD. $p<0.05$ was considered statistically significant.

Results

Exposure to Mechanical Ventilation Induced Autophagy and c-Src Signaling Activation

The critical role of autophagy in mechanical ventilation-induced lung inflammatory injury has been clearly shown in previous studies[19,20]. On the other hand, it is well recognized that c-Src plays versatile functions in macrophage-mediated innate immunity. Therefore, we explored the effects of autophagy on c-Src signaling pathway. To this end, mice were subjected to a lower or higher lung stretch induced by mechanical ventilation with low (7 mL/kg) or high (28 mL/kg) tidal volume, respectively, prior to the assessment of activation of autophagy and c-Src. As we all know, lapidated LC3-II has been identified as a constitutive component on the autophagosomal membrane and is decreased due to the absence of autophagy, thereby being deemed as a specific autophagy hallmark[12].

As shown in Figure 1A, the LC3-II expression was markedly augmented in mice subject-

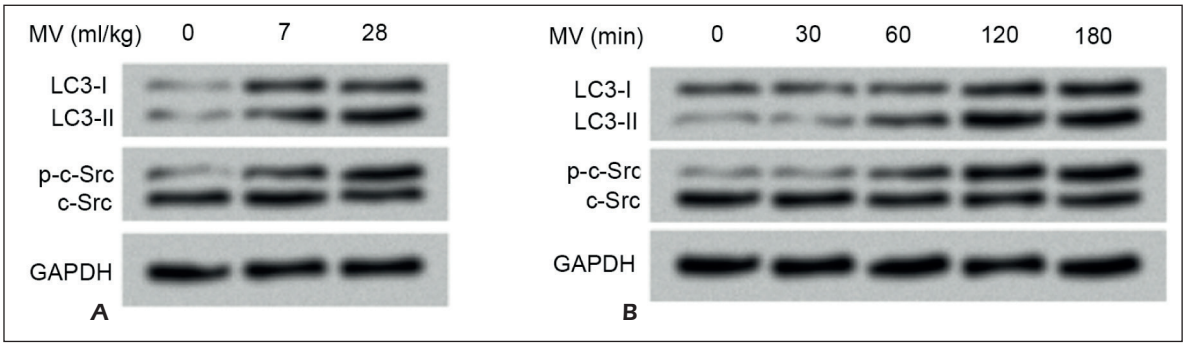

Figure 1. LC3-II expression and c-Src phosphorylation were dramatically enhanced in a tidal volume- and duration-dependent manner. **A**, Mice were subjected to 2 h of mechanical ventilation with low (7 mL/kg) or high (28 mL/kg) tidal volume, followed by western blot analysis. **B**, Mice were exposed to mechanical ventilation with a high tidal volume (28 mL/kg) for 3 h. Protein expression was measured at different time points.

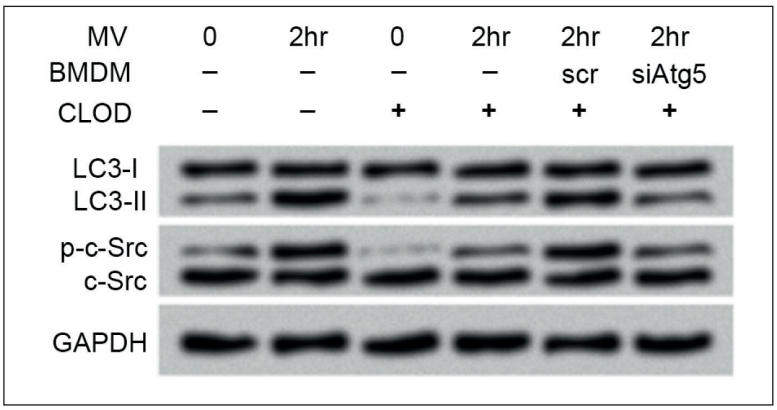

Figure 2. Atg5 knockdown inhibited autophagy and c-Src activation. Mice AMs were depleted with clodronate liposomes (CLOD). AM-depleted mice were replete with BMDMs isolated from other donor mice and transfected with a scrambled siRNA (scr) or Atg5 siRNA (siAtg5). Western blot was performed to measure the protein expression in lysates from lung homogenates of mice with or without mechanical ventilation (28 mL/kg) for 2 h.

ed to 2 h of high tidal volume ventilation, while exposure to a low tidal volume ventilation for the same duration did not result in an evident increase in LC3-II expression level, in agreement with the previous study[20]. For further investigation of the effects of mechanical ventilation on autophagy, the LC3-II level was assessed at different time points during high tidal volume ventilation. The LC3-II protein expression was aggrandized within 20 min and maintained at a maximal level for at least 2 h. Likewise, high-tidal-volume ventilation also resulted in a remarkable increase in the expression of phosphorylated c-Src in a time-dependent and tidal volume-dependent manner (Figure 1A and B). These indicated that c-Src activation was enhanced following high-tidal-volume ventilation, which was also related to autophagy initiation.

Genetic Inhibition of Autophagy Blocks c-Src Activation Following Mechanical Ventilation

It seems that mechanical ventilation-induced autophagy occurs mainly in pulmonary macrophages[20]. Thus, BMDMs were transfected with Atg5 siRNA and administered to AM-depleted mice to generate an autophagy-deficient phenotype. Also, lipidation of LC3 was assessed by Western blot analysis to visualize the autophagy status following high-tidal-volume ventilation in AM-depleted mice carrying Atg5 siRNA-transfected BMDMs. As expected, the augmented LC3-II level induced by mechanical ventilation was significantly attenuated in the lung homogenates of these mice (Figure 2). Moreover, the phosphorylation of c-Src protein was markedly decreased in lungs of the autophagy-deficient mice subjected to high-tidal-volume ventilation compared to normal lungs (Figure 2). These indicated that the impairment of autophagy in macrophages led to a dramatic decrease in the activation of the c-Src signaling pathway.

Impairment of c-Src Activation Attenuated Lung Inflammatory Response Caused by Injurious Ventilation

We attempted to further elucidate the role of c-Src pathway activation in autophagy-mediated lung inflammatory injury during mechanical ventilation. To this end, the c-Src pathway was blocked by pre-treatment of mice with a specific kinase inhibitor (1 μg/kg PP2) for 30 min, prior to the evaluation of the severity and extent of lung injury. As shown in Figure 3A, the LC3-II expression did not change due to c-Src signaling abolishment. However, the polymorphonuclear neutrophils (PMN, Figure 3B) and total cell counts (Figure 3C) in BAL fluid, as well as lung injury scores (Figure 3D), showed that injurious ventilation resulted in a considerable increase in the infiltration or aggregation of neutrophils and lung edema formation in control mice (without c-Src inhibition), which was markedly attenuated by PP2 or AM-abolishment (Figure 3B-3D).

c-Src blockage ameliorated lung inflammatory response but did not alter LC3 lipidation and, in addition, ventilation-induced autophagy enhanced c-Src activation, whereas autophagy depletion suppressed phosphorylated c-Src expression. These all together suggest that autophagy caused inflammatory lung injury *via* regulating c-Src pathway activation during mechanical ventilation.

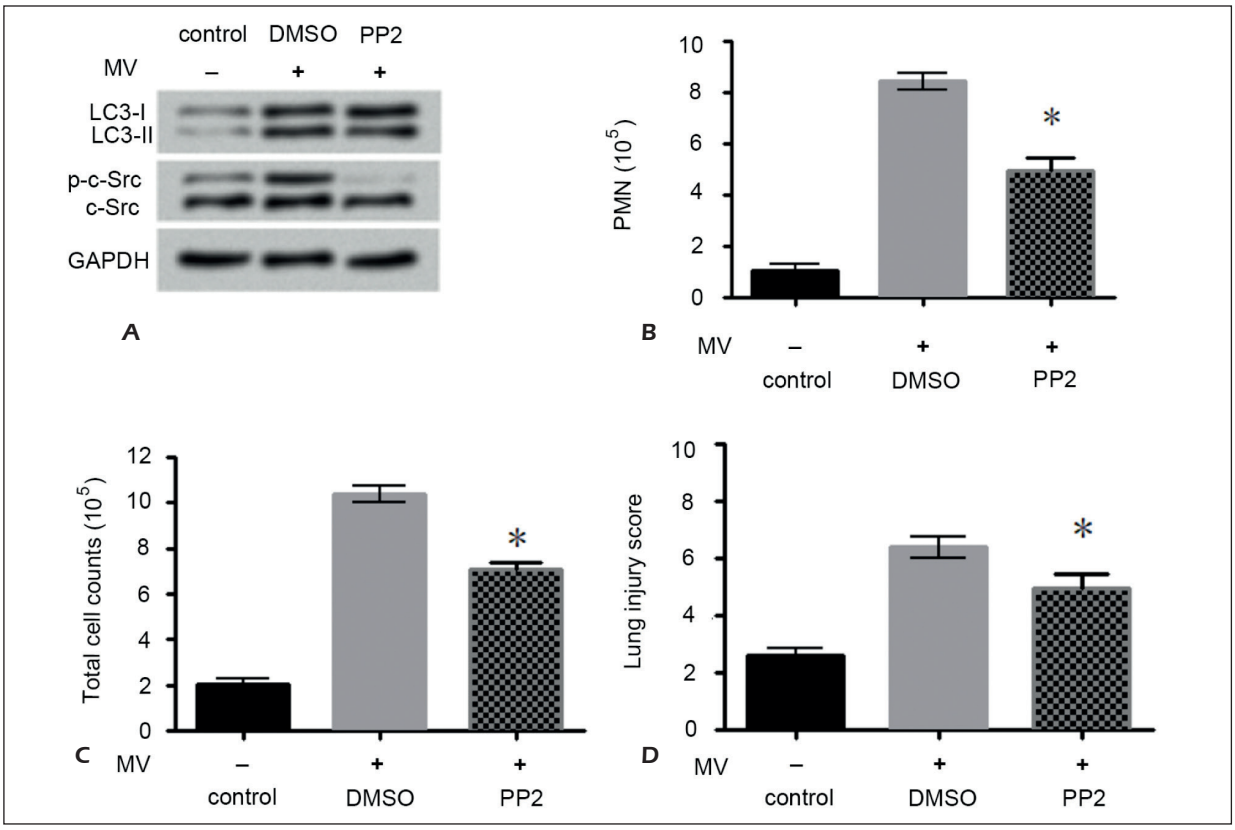

Figure 3. c-Src pathway blockade did not impair autophagy but attenuated lung inflammatory response. **A**, The expression of autophagy-related proteins was visualized by Western blot assay. Inflammatory response was represented by PMN (**B**) and total cell counts (**C**) in BAL fluid. **D**, Lung injury was also evaluated.

Discussion

Src is a non-receptor tyrosine kinase originally identified in 1967, which has been reported to participate in cross-talk between numerous signaling pathways, such as Ras/Raf/MEK, PI3K/Akt, JAK/STAT, and EGF receptor pathways. Src directly tyrosine-phosphorylates its target proteins on activation sites, and Src activity is modulated by the structural alterations resulting from the auto-phosphorylation or de-phosphorylation on its own tyrosine residues. It has been well established that Src kinases regulate cell proliferation, adhesion, differentiation as well as survival and, in particular, Src plays a crucial role as a proto-oncogene in the occurrence and progression of tumors[21]. Dohn et al[22] have demonstrated that c-Src is also implicated in inflammation-related signaling pathways, thereby mediating various immunologic events, including immune cell adhesion, migration, recruitment, and phagocytosis.

By using specific pharmacologic inhibitor or siRNA, researchers have demonstrated that Src is involved in the process of PMN migration, which is induced by alveolar macrophages that have been proposed to be the only resident effector cells to satisfy this function during acute lung injury[23]. There is increasing evidence that integrin-FAK-Src signaling pathways advance cell cycle progression and gene transcription of immune cells, especially macrophages[24]. Furthermore, c-Src is responsible for inflammatory responses to acute lung injury in mice incited by LPS. In detail, upon LPS incitement, c-Src is auto-phosphorylated and triggers inflammatory signaling cascades including NF-κB and TNF-α activation, increase in total proteins in BAL fluid, and neutrophil recruitment. These all can be suppressed by the inhibition of c-Src activation[25]. In our previous works, we found that c-Src tyrosine kinase pathway was involved in ventilation-induced lung inflammatory injury through regulating the degradation of p120-catenin and occluding.

It has been well established that a mechanical stress can induce an extensive autophagic response in the lung. Given that autophagy predominantly takes place in alveolar macrophages during ventilation-induced lung injury[20], and that c-Src pathway is involved in the improved development of macrophages, important questions in deciphering the mechanisms underlying their role in immune and inflammatory responses to injurious ventilation include: (1) Does the autophagy-mediated inflammation depend on c-Src phosphorylation? (2) Will autophagy depletion dampen c-Src activation and the subsequent pulmonary inflammatory lesions?

Mice lacking the critical gene Atg5 leads to a thorough loss of autophagic response to that they cannot survive after the postnatal period. Thus, alveolar macrophages were eliminated from mice lungs and then BMDMs transfected with Atg5 siRNA were administered to the AM-depleted mice to study the effects of autophagy impairment on c-Src activation. As shown in Figure 2, the autophagy-deficient phenotype resulted in a markedly compromised augment in c-Src phosphorylation following high-tidal-volume ventilation, which indicated that impairment in alveolar autophagy could block c-Src signaling activation. Therefore, we propose that c-Src pathway in the lung can also be activated by autophagy, besides the regulation of c-Src activity by itself through auto-phosphorylation or by other tyrosine kinases or phosphatases.

On the other hand, treatment with c-Src inhibitor PP2 ameliorated inflammatory response triggered by injurious ventilation, including increased PMN and total cell counts in BAL fluid, lung injury scores, and exacerbated lung edema (Figure 3). c-Src abolishment was responsible for the inflammatory response although the autophagic status was not altered, suggesting that the autophagy-mediated inflammatory injury is dependent upon c-Src activation.

Conclusions

We indicated that ventilation with high-tidal volume induced a substantial increase in autophagy and c-Src activation, whilst underlining the pivotal role of c-Src in the autophagy-mediated inflammatory cascades in lung injury, which implies that c-Src interruption may represent a promising preventive therapy to ameliorate the pulmonary inflammatory injury following mechanical ventilation.

Funding

This work was supported by Shandong Provincial Natural Science Foundation of China (ZR2017PH045) and the Young teacher Foundation of Jining Medical College (JY2016KJ039Y).

Conflict of Interests

The authors declare that they haven't a conflict of interest.

References

1) Ngiam N, Kavanagh BP. Ventilator-induced lung injury: the role of gene activation. Curr Opin Crit Care 2012; 18: 16-22.
2) Liu D, Geng Z, Zhu W, Wang H, Chen Y, Liang J. 15-deoxy-Delta(1)(2),(1)(4)-prostaglandin J(2) ameliorates endotoxin-induced acute lung injury in rats. Chin Med J (Engl) 2014; 127: 815-820.
3) Li H, Wu Z, Feng D, Gong J, Yao C, Wang Y, Yuan S, Yao S, Shang Y. BML-111, a lipoxin receptor agonist, attenuates ventilator-induced lung injury in rats. Shock 2014; 41: 311-316.
4) Hess DR, Thompson BT, Slutsky AS. Update in acute respiratory distress syndrome and mechanical ventilation 2012. Am J Respir Crit Care Med 2013; 188: 285-292.
5) Tremblay LN, Miatto D, Hamid Q, Govindarajan A, Slutsky AS. Injurious ventilation induces widespread pulmonary epithelial expression of tumor necrosis factor-alpha and interleukin-6 messenger RNA. Crit Care Med 2002; 30: 1693-1700.
6) Zhang H, Downey GP, Suter PM, Slutsky AS, Ranieri VM. Conventional mechanical ventilation is associated with bronchoalveolar lavage-induced activation of polymorphonuclear leukocytes: a possible mechanism to explain the systemic consequences of ventilator-induced lung injury in patients with ARDS. Anesthesiology 2002; 97: 1426-1433.
7) Dunn I, Pugin J. Mechanical ventilation of various human lung cells in vitro: identification of the macrophage as the main producer of inflammatory mediators. Chest 1999; 116: 95S-97S.
8) Duan YT, Bi KY, Ma YS. PKC delta gene can induce macrophages to release inflammatory factors against Mycobacterium tuberculosis infection. Eur Rev Med Pharmacol Sci 2018; 22: 4228-4237.
9) McKechnie SR, Drummond GB. Cytokines, neurokines or both? Mixed mechanisms of mechanical lung injury. J Physiol 2010; 588: 1813-1814.
10) Patel AS, Morse D, Choi AM. Regulation and functional significance of autophagy in respiratory cell biology and disease. Am J Respir Cell Mol Biol 2013; 48: 1-9.
11) Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, Green-Thompson ZW, Jimenez-Sanchez M, Korolchuk VI, Lichtenberg M, Luo S, Massey DC, Menzies FM, Moreau K, Narayanan U, Renna M, Siddiqi FH, Underwood BR, Winslow AR, Rubinsztein DC. Regulation of mammalian autophagy in physiology and pathophysiology. Physiol Rev 2010; 90: 1383-1435.

12) Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, Yoshimori T. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 2000; 19: 5720-5728.
13) Thurston TL, Wandel MP, von Muhlinen N, Foeglein A, Randow F. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 2012; 482: 414-418.
14) Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, Sadoshima J. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ Res 2007; 100: 914-922.
15) Mizushima N. The pleiotropic role of autophagy: from protein metabolism to bactericide. Cell Death Differ 2005; 12 Suppl 2: 1535-1541.
16) Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 2007; 315: 1398-1401.
17) Conway MA, Kefala K, Wilkinson TS, Moncayo-Nieto OL, Dhaliwal K, Farrell L, Walsh TS, Mackenzie SJ, Swann DG, Andrews PJ, Anderson N, Govan JR, Laurenson IF, Reid H, Davidson DJ, Haslett C, Sallenave JM, Simpson AJ. Diagnostic importance of pulmonary interleukin-1beta and interleukin-8 in ventilator-associated pneumonia. Thorax 2010; 65: 201-207.
18) Zhao T, Zhao H, Li G, Zheng S, Liu M, Gu C, Wang Y. Role of the PKCalpha-c-Src tyrosine kinase pathway in the mediation of p120-catenin degradation in ventilator-induced lung injury. Respirology 2016; 21: 1404-1410.
19) Lopez-Alonso I, Aguirre A, Gonzalez-Lopez A, Fernandez AF, Amado-Rodriguez L, Astudillo A, Batalla-Solis E, Albaiceta GM. Impairment of autophagy decreases ventilator-induced lung injury by blockade of the NF-kappaB pathway. Am J Physiol Lung Cell Mol Physiol 2013; 304: L844-L852.
20) Zhang Y, Liu G, Dull RO, Schwartz DE, Hu G. Autophagy in pulmonary macrophages mediates lung inflammatory injury via NLRP3 inflammasome activation during mechanical ventilation. Am J Physiol Lung Cell Mol Physiol 2014; 307: L173-L185.
21) Zachary I. VEGF signalling: integration and multi-tasking in endothelial cell biology. Biochem Soc Trans 2003; 31: 1171-1177.
22) Dohn MR, Brown MV, Reynolds AB. An essential role for p120-catenin in Src- and Rac1-mediated anchorage-independent cell growth. J Cell Biol 2009; 184: 437-450.
23) Wang Z, Rui T, Yang M, Valiyeva F, Kvietys PR. Alveolar macrophages from septic mice promote polymorphonuclear leukocyte transendothelial migration via an endothelial cell Src kinase/NADPH oxidase pathway. J Immunol 2008; 181: 8735-8744.
24) Thomas SM, Soriano P, Imamoto A. Specific and redundant roles of Src and Fyn in organizing the cytoskeleton. Nature 1995; 376: 267-271.
25) Lee HS, Moon C, Lee HW, Park EM, Cho MS, Kang JL. Src tyrosine kinases mediate activations of NF-kappaB and integrin signal during lipopolysaccharide-induced acute lung injury. J Immunol 2007; 179: 7001-7011.