

## Secretion of related factors and AMPK/PGC-1 $\alpha$ signal pathway by alveolar macrophages in viral acute lung injury

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### ABSTRACT

This study was to investigate the secretion of related factors by alveolar macrophages and AMPK/PGC-1  $\alpha$  signal pathway in viral acute lung injury (VALI) patients. 30 SD rats were randomly divided into blank control group (BCG) and VALI group (VALIG) with 15 rats in each group. The model of VALIG was induced by polyI: C and the BCG was given the same amount of normal saline. The IL-6, IL-8 and TNF- $\alpha$  levels in bronchoalveolar lavage fluid (BALF) were detected by ELISA. The protein, surface markers of alveolar macrophages, and related mRNA expression were detected by Western blot, ELISA, and qRT-PCR. IL-8, IL-6, TNF- $\alpha$ , MUC5AC and TLR4 levels in VALIG were raised than those in BCG ( $P < 0.05$ ), while the AQP5 level was reduced than that in the BCG ( $P < 0.05$ ). The Bcl-2 protein level in the VALIG was reduced than that in the BCG ( $P < 0.05$ ), while the Caspase3 protein level was raised than that in the BCG ( $P < 0.05$ ). The AMPK and PGC-1  $\alpha$  mRNA and protein expression level in the VALIG rat lung tissue was lower than that of the BCG ( $P < 0.05$ ) VALIG is related to inflammatory damage, activation of alveolar macrophages, and secretion of related factors by alveolar macrophages. This may be related to AMPK/PGC-1  $\alpha$  signal pathways.

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### Introduction

Acute lung injury (ALI) is a kind of lung parenchyma injury disease, which refers to severe hypoxemia, respiratory distress caused by various causes, and even can not be corrected by routine inhalation of oxygen. more serious, it is called acute respiratory distress syndrome (ARDS) (1). Respiratory virus infection, gastric acid inhalation, sepsis and other pathogenic factors can lead to ALI, including influenza A virus, avian influenza virus, coronavirus and novel coronavirus, which caused the global pandemic (2). Viral ALI (VALI) is usually caused by diffuse capillary endothelial and alveolar epithelial injury after severe injury. Patients may have chest pain, chest tightness, shortness of breath and shortness of breath. If it is not controlled in time, the condition can be gradually aggravated (3). Severe cases may result in hemoptysis, respiratory distress, respiratory failure, hypoxemia, hypercapnia, lethargy, coma, and even death, which pose a serious threat to the patient's life and health (4). At present, the clinical treatment of ARDS is mainly based on basic treatment and symptomatic support therapy. Although it can achieve a certain effect, it is not good for the progression of pulmonary inflammation. Alveolar macrophages are a kind of inflammatory cells widely found on the surface of bronchi and alveoli. It has been found that excessive release of inflammatory cytokines by alveolar macrophages is the key to lung tissue injury in patients with VALI (5). Adenylate-activated protein kinase/peroxisome proliferator-activated

gamma receptor coactivator 1- $\alpha$  (AMPK/PGC-1  $\alpha$ ) signal pathway is a widely used signal transduction pathway in the human body, which plays a critical role in regulating cell function (6). This study aimed to explore the relationship between the secretion of related factors by alveolar macrophages and the AMPK/PGC-1  $\alpha$  signal pathway in patients with VALI, so as to provide an experimental basis for clinical containment of VALI.

### Materials and Methods

#### Materials and reagents

We purchased 30 SD male rats from our hospital's animal management center, aged 6-8 weeks and weighing 180-220 g. Trizol RNA extraction kit and reverse transcription kit, interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Biyuntian Co., Ltd.); AMPK, PGC-1  $\alpha$ , Caspase3, Bcl-2 antibody (British Abcam Biotechnology Co., Ltd.); Immunohistochemical sheep anti-rabbit second antibody (Shanghai Biyuntian Co., Ltd.).

#### Establish an animal model

30 SD rats were randomly divided into a blank control group (BCG) and a VALI group (VALIG) with 15 rats in each group. Using the model of VALI induced by polyI: C, the rats were anesthetized, and the mice were fixed on the mouse plate with a leather band through the upper anterior incisor, the pipette was inserted into the oropharynx, and

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50  $\mu$ l of polyI: C liquid was injected slowly, and all the liquid was inhaled after at least 5 breathing cycles. The BCG was given the same amount of inhaled normal saline.

### Sampling and sample preparation

The rats were anesthetized with 10% chloral hydrate, the thorax was opened, the sterile PBS solution was injected into the tracheal intubation, and the bronchoalveolar lavage fluid (BALF) was collected for examination. Cut the chest and remove the right lung tissue and transfer it to the refrigerator at -80 °C for examination.

### Detect the level of the biochemical index

BALF uses a separator to separate 20min according to 3000r/min, and the supernatant is reserved for detection. Part of the supernatant is strictly in accordance with the instructions of the ELISA detection kit to detect the levels of IL-6, IL-8 and TNF- $\alpha$ .

### Expression of surface markers of alveolar macrophages

Another part of BALF was separated from 20min by 3000r/min in a separator, and the lower supernatant was reserved for examination. After collecting the sputum cells at the bottom of the test tube after centrifugation, the macrophages in the sputum were separated by Rosette Sep cell separation. After the macrophages were cultured, the mRNA was extracted, and reverse transcribed into cDNA by One Step Prime Script miRNA cDNA synthesis kit. MiRNA real-time PCR of Toll-like receptor 4 (TLR4), mucin 5AC (MUC5AC) and aquaporin 5 (AQP5) were performed by fluorescence quantitative PCR kit, and the cycle was completed according to the instructions of the kit. After the reaction is completed, the expression is calculated in the software.

### Detection of mRNA expression in lung tissue

After mRNA extraction, reverse transcribed into cDNA. Real-time PCR of mRNA was performed by miRNA fluorescence quantitative PCR detection kit, and the cycle was

completed according to the instructions of the kit. Calculate the relative expression of mRNA in the software after the reaction is completed.

### Western blot

After the lung tissue was homogenized at 4 °C to make 10% homogenate, the supernatant was centrifuged to be examined, BCA method was used to determine the protein concentration, gel preparation, electrophoretic 90min, gel cutting, membrane transfer 90min, milk sealing, washing and incubation with antibodies, development, and the results were analyzed by Bio-Rad image laboratory software.

### Statistical analysis

SPSS20.0 was used for statistical analysis, and the measurement data were expressed by mean  $\pm$  standard deviation ( $\bar{x}\pm s$ ). T-test was used for group comparison.

### Results

#### Comparison of related inflammatory factors secreted by alveolar macrophages

IL-8, IL-6 and TNF- $\alpha$  levels in VALIG were raised than those in BCG ( $P<0.05$ ) (Table 1).

#### Expression of surface markers of alveolar macrophages

MUC5AC and TLR4 levels in the VALIG were raised than those in the BCG ( $P<0.05$ ), meanwhile AQP5 level was reduced than in the BCG ( $P<0.05$ ) (Table 2).

#### Protein expression of Caspase3 and Bcl-2 in rat alveolar macrophages

The expression of Bcl-2 protein in the VALIG was reduced than that in the BCG ( $P<0.05$ ), while Caspase3 was raised than in the BCG ( $P<0.05$ ), (Table 3).

#### Relative expression of AMPK and PGC-1 $\alpha$ mRNA in rat lung tissue

AMPK and PGC-1  $\alpha$  mRNA levels in the lung tissue

**Table 1.** Related inflammatory factors secreted.

Group	n	IL-8( $\mu\text{g}\cdot\text{L}^{-1}$ )	IL-6 ( $\mu\text{g}\cdot\text{L}^{-1}$ )	TNF- $\alpha$ ( $\text{ng}\cdot\text{L}^{-1}$ )
BCG	15	2.76 $\pm$ 0.29	97.86 $\pm$ 11.28	50.71 $\pm$ 8.22
VALIG	15	3.45 $\pm$ 0.74	150.37 $\pm$ 16.09	121.41 $\pm$ 13.14
<i>t</i>		3.362	10.350	17.667
<i>P</i>		0.002	0.000	0.000

**Table 2.** Surface markers of rat alveolar macrophages.

Group	n	AQP5	MUC5AC	TLR4
BCG	15	6.76 $\pm$ 2.29	1.86 $\pm$ 0.68	0.81 $\pm$ 0.32
VALIG	15	3.34 $\pm$ 1.11	4.28 $\pm$ 1.04	2.84 $\pm$ 1.26
<i>t</i>		5.205	-7.543	-6.048
<i>P</i>		0.000	0.000	0.000

**Table 3.** Protein expression of Caspase3 and Bcl-2 in rat alveolar macrophages.

Group	n	Bcl-2	Caspase3
BCG	15	0.76 $\pm$ 0.23	0.51 $\pm$ 0.13
VALIG	15	0.35 $\pm$ 0.12	0.85 $\pm$ 0.23
<i>t</i>		6.121	4.982
<i>P</i>		0.000	0.000

**Table 4.** AMPK and PGC-1  $\alpha$  mRNA levels in rat lung tissue.

Group	n	AMPK	PGC-1 $\alpha$
BCG	15	1.65 $\pm$ 0.40	1.76 $\pm$ 0.43
VALIG	15	1.24 $\pm$ 0.32	0.83 $\pm$ 0.35
<i>t</i>		3.010	6.497
<i>P</i>		0.004	0.000

**Table 5.** AMPK and PGC-1  $\alpha$  protein levels in rat lung tissue.

Group	n	AMPK	PGC-1 $\alpha$
BCG	15	0.57 $\pm$ 0.08	0.52 $\pm$ 0.09
VALIG	15	0.28 $\pm$ 0.04	0.30 $\pm$ 0.03
<i>t</i>		12.557	5.524
<i>P</i>		0.000	0.000

of the VALIG were reduced than that of the BCG ( $P < 0.05$ ) (Table 4).

### The expression level of AMPK and PGC-1 $\alpha$ protein in rat lung tissue

AMPK and PGC-1  $\alpha$  protein levels in the lung tissue of rats in VALIG were reduced than those in BCG ( $P < 0.05$ ) (Table 5).

### Discussion

VALIG refers to acute and progressive hypoxic respiratory failure caused by respiratory viruses, pulmonary gas exchange disorders and pulmonary mechanical abnormalities, more seriously referred to as ARDS (7). The pathogenesis of VALIG is a process of diffuse lung injury. Irregular alveolar inflammatory substances can be exudated from multiple lung fields in the bilateral lung tissue of patients, which is a common clinical critical disease. Patients often show hypoxemia, decreased transmittance of both lungs, increased intrapulmonary shunt and physiologically ineffective cavity, and decreased pulmonary compliance, which can lead to respiratory failure, multiple organ dysfunction and even death (8). Although various new technologies, including ventilator support technology, blood purification technology and the birth of new antiviral drugs, continue to emerge and achieve certain therapeutic effects, some patients still have a high case fatality rate (9). Therefore, it is urgent to find a treatment that can reduce the clinical symptoms, prolong the survival rate and reduce the mortality in patients with VALI/ARDS. This study aimed to explore the relationship between the secretion of related factors by alveolar macrophages and the AMPK/PGC-1  $\alpha$  signal pathway in patients with VALI, so as to provide an experimental basis for clinical containment of VALIG (10).

The pathogenesis of VALI has not been fully explained, but recent studies have found that the local inflammatory response of the lung is out of control and the injury of pulmonary epithelial and endothelial cells mediated by it leads to the increase of pulmonary vascular permeability. the decrease of pulmonary surfactant is the main pathogenesis of VALI (11). Various pathogenic factors inside and outside the lung cause acute uncontrolled inflammatory reactions in lung tissue, resulting in alveolar capillary injury, increased permeability and pulmonary edema, which lead to progressive dyspnea and hypoxemia are the main pathophysiological changes of VALI (12). Alveolar

macrophages are multifunctional immune cells, which have many biological functions, such as phagocytosis, removal of microorganisms and cell fragments, immune surveillance, repair of injury, maintenance of homeostasis, promotion of inflammatory regression and so on (13). TGF- $\beta$ 1, IL-6 and TNF- $\alpha$  are the main inflammatory cytokines and fibrosis regulatory factors, among which TNF- $\alpha$  is an endogenous cytokine, which can promote the release of proinflammatory factors by activating middle lymphocytes, macrophages, neutrophils and other immune inflammatory cells. IL-6 induces an acute inflammatory response by promoting the activation of lymphocytes in the blood (14). We found the levels of IL-8, IL-6 and TNF- $\alpha$  in the VALIG were raised than those in the BCG. It is suggested that VALI infection is related to inflammatory injury.

In addition, macrophages can mediate phagocytosis through pattern recognition receptors on the cell surface. The changes in surface markers of alveolar macrophages such as AQP5, MUC5AC and TLR4 are closely related to the progression of pulmonary inflammatory injury (15). Bacterial proteins can activate macrophages through TLRs and promote the production of TGF- $\beta$  1, IL-6 and TNF- $\alpha$ , which can mediate the accumulation of inflammatory cells, induce the killing function of macrophages and eliminate pathogens. MUC5AC is a mucin produced by respiratory epithelial cells, which is most sensitive to pathophysiological changes in the lung and can be highly induced. the increase of its level can lead to excessive secretion of mucin, which provides a good medium for virus reproduction, thus aggravating airway inflammation in patients with VALI (16); AQP5 is an important water transport channel in the airway and lung tissue. The decrease in its level can lead to too little mucus secretion, which increases the concentration of mucin and provides a good medium for virus reproduction (17). The results showed that the expression levels of MUC5AC and TLR4 in the VALIG were raised than those in the BCG, while the expression level of AQP5 in the VALIG was reduced to that in the BCG. It is suggested that VALI is related to the activation of alveolar macrophages. In addition, it has been found that apoptosis of alveolar macrophages is an important link in the VALI progression. In many cases, such as endogenous or exogenous stimulation, alveolar macrophages can be induced to enter the apoptosis process (18). The increase in the number of apoptosis of alveolar macrophages not only leads to the non-phagocytosis of apoptotic neutrophils but also causes the release of inflammatory mediators and oxidative stress-related products to

enter the lung tissue, further aggravating the injury of lung tissue and promoting the development of patients with VALI (19). Caspase3 is the most representative pro-apoptotic molecule, and Bcl-2 is an anti-apoptotic molecule, which is closely related to the apoptosis of alveolar macrophages (20). The results showed that the Bcl-2 protein level in the VALIG was reduced to that in the BCG, and the Caspase3 protein level in the VALIG was raised more than that in the BCG.

AMPK/PGC-1  $\alpha$  signal pathway is a widely used signal transduction pathway in the human body, which plays a critical role in regulating cell differentiation, migration, proliferation, and apoptosis (21). AMPK belongs to serine/threonine protein kinase, which is a critical factor in regulating cell energy homeostasis and inflammation. It is expressed in various metabolic organs and tissues. It can be activated by various stimuli by sensing changes in the state of cell energy metabolism, thus affecting multiple links of cell material metabolism to coordinate body metabolism and energy balance (22). When there is an imbalance between metabolism and energy, activation of AMPK can regulate the expression of downstream malonyl CoA and lipid synthesis genes through phosphorylation, regulate the biosynthesis of fatty acids, thus inhibiting inflammation and oxidative stress, and restore the energy balance of the body (23). PGC1- $\alpha$  can activate the transcription factor of mitochondrial DNA, mitochondrial transcription factor A (TFAM). The activated TFAM can pass through the mitochondrial membrane into the mitochondrial matrix to bind to mitochondrial DNA and form a transcription initiation complex with mitochondrial transcription factor B (TFBM) and mitochondrial RNA polymerase, thus regulating mitochondrial biogenesis and function (24). We found that the relative expression of AMPK and PGC-1  $\alpha$  mRNA in the lung tissue of rats in the VALIG was reduced than that in the BCG. The AMPK and PGC-1  $\alpha$  expression levels in the lung tissue of rats in the VALIG were reduced than those in the BCG.

To sum up, VALI is related to inflammatory injury, activation of alveolar macrophages and secretion of related factors by alveolar macrophages, and may be related to the regulation of the AMPK/PGC-1  $\alpha$  signal pathway.

## References

- Feng ZY, Jing Z, Li Q, ChuLX, JiangYX, ZhangXB, YanL, LiuYH, JiangJ, XuP, ChenQ, WangM, YangH, ZhouGR, JiangXC, ChenXY, XiaHP. Exosomal STIMATE derived from type II alveolar epithelial cells controls metabolic reprogramming of tissue-resident alveolar macrophages. *Theranostics* 2023; 13(3): 991-1009. <https://doi.org/10.7150/thno.82552>
- Xu MM, Kang JY, Ji S, Wei YY, Wei SL, Ye JJ, Wang YG, Shen JL, Wu HM, Fei GH. Melatonin Suppresses Macrophage M1 Polarization and ROS-Mediated Pyroptosis via Activating ApoE/LDLR Pathway in Influenza A-Induced Acute Lung Injury. *Oxid Med Cell Longev* 2022; 2022: 2520348. <https://doi.org/10.1155/2022/2520348>
- Ohya M, Tateishi A, Matsumoto Y, Satomi H, Kobayashi M. Bystander CD8<sup>+</sup>T cells may be involved in the acute phase of diffuse alveolar damage. *Virchows Arch* 2023; 482(3): 605-613. <https://doi.org/10.1007/s00428-023-03521-w>
- Wang X, Zhou L, Ye S, Liu S, Chen L, Cheng Z, Huang Y, Wang B, Pan M, Wang D, Wang L, Lei Z, Im YJ, Li X. rFGF4 alleviates lipopolysaccharide-induced acute lung injury by inhibiting the TLR4/NF- $\kappa$ B signaling pathway. *Int Immunopharmacol* 2023; 117: 109923. <https://doi.org/10.1016/j.intimp.2023.109923>
- BondaWLM, Fournet M, Zhai RY, LutzJ, BlondonnetR, BourgneC, LeclairC, Saint-BéatC, TheilliereC, BelvilleC, BouverierD, BlanchonL, BergerM, SapinV, JabaudonM. Receptor for advanced glycation end-products promotes activation of alveolar macrophages through the NLRP3 Inflammasome/TXNIP axis in acute lung injury. *Int J Mol Sci* 2022; 23(19): 11659. <https://doi.org/10.3390/ijms231911659>
- Gong L, Shen Y, Wang S, Wang X, Ji H, Wu X, Hu L, Zhu L. Nuclear SPHK2/S1P induces oxidative stress and NLRP3 inflammasome activation via promoting p53 acetylation in lipopolysaccharide-induced acute lung injury. *Cell Death Discov* 2023; 9(1): 12. <https://doi.org/10.1038/s41420-023-01320-5>
- Liu C, Liu YH, Xi L, He Y, Liang YM, Mak JCW, Mao SR, Wang ZP, ZhengY. Interactions of inhaled liposome with macrophages and neutrophils determine particle biofate and anti-inflammatory effect in acute lung inflammation. *ACS Appl Mater Interfaces* 2023; 15(1): 479-493. <https://doi.org/10.1021/acsami.2c17660>
- Xu X, Liu X, Dong X, Yang Y, Liu L. MIR-199a-3p-regulated alveolar macrophage-derived secretory autophagosomes exacerbate lipopolysaccharide-induced acute respiratory distress syndrome. *Front Cell Infect Microbiol* 2022; 12: 1061790. <https://doi.org/10.3389/fcimb.2022.1061790>
- Wu X, Wu L, Wu Y, Chen W, Chen J, Gong L, Yu J. Heme oxygenase-1 ameliorates endotoxin-induced acute lung injury by modulating macrophage polarization via inhibiting TXNIP/NLRP3 inflammasome activation. *Free Radic Biol Med* 2023; 194: 12-22. <https://doi.org/10.1016/j.freeradbiomed.2022.11.032>
- Hu Q, Yao J, Wu X, Li J, Li G, Tang W, Liu J, Wan M. Emodin attenuates severe acute pancreatitis-associated acute lung injury by suppressing pancreatic exosome-mediated alveolar macrophage activation. *Acta Pharm Sin B* 2022; 12(10): 3986-4003. <https://doi.org/10.1016/j.apsb.2021.10.008>
- Wang K, Rong G, Gao Y, Wang M, Sun J, Sun H, Liao X, Wang Y, Li Q, Gao W, Cheng Y. Fluorous-Tagged Peptide Nanoparticles Ameliorate Acute Lung Injury via Lysosomal Stabilization and Inflammation Inhibition in Pulmonary Macrophages. *Small* 2022; 18(40): e2203432. <https://doi.org/10.1002/sml.202203432>
- Gopalakrishnan A, Joseph J, Shirey KA, Keegan AD, Boukhvalova MS, Vogel SN, Blanco JCG. Protection against influenza-induced Acute Lung Injury (ALI) by enhanced induction of M2a macrophages: possible role of PPAR $\gamma$ /RXR ligands in IL-4-induced M2a macrophage differentiation. *Front Immunol* 2022; 13: 968336. <https://doi.org/10.3389/fimmu.2022.968336>
- Cong Z, Yang C, Cheng Z, Wu C, Zhao F, Shen Z, Xiao H, Zhu X.  $\alpha_1$ -adrenoceptor stimulation ameliorates lipopolysaccharide-induced lung injury by inhibiting alveolar macrophage inflammatory responses through NF- $\kappa$ B and ERK1/2 pathway in ARDS. *Front Immunol* 2023 Jan 6; 13: 1090773. <https://doi.org/10.3389/fimmu.2022.1090773>
- Jerkic M, Szaszi K, Laffey JG, Rotstein O, Zhang H. Key Role of Mesenchymal Stromal Cell Interaction with Macrophages in Promoting Repair of Lung Injury. *Int J Mol Sci* 2023; 24(4): 3376. <https://doi.org/10.3390/ijms24043376>
- Wang WB, Li JT, Hui Y, Shi J, Wang XY, Yan SG. Combination of pseudoephedrine and emodin ameliorates LPS-induced acute lung injury by regulating macrophage M1/M2 polarization through the VIP/cAMP/PKA pathway. *Chin Med* 2022; 17(1): 19. <https://doi.org/10.1186/s13020-021-00562-8>
- Yin S, Ding M, Fan L, Yu X, Liang Z, Wu L, Gao Z, Lin L, Chen Y. Inhibition of inflammation and regulation of AQPs/ENaCs/Na<sup>+</sup>-K<sup>+</sup>-ATPase mediated alveolar fluid transport by total flavonoids extracted from *Nervilia fordii* in lipopolysaccharide-

- induced acute lung injury. *Front Pharmacol* 2021; 12: 603863. <https://doi.org/10.3389/fphar.2021.603863>
17. Bhattacharya SS, Yadav B, Yadav E, Hus A, Yadav N, Kaur P, Rosen L, Jandarov R, Yadav JS. Differential modulation of lung aquaporins among other pathophysiological markers in acute (Cl<sub>2</sub> gas) and chronic (carbon nanoparticles, cigarette smoke) respiratory toxicity mouse models. *Front Physiol* 2022; 13: 880815. <https://doi.org/10.3389/fphys.2022.880815>
18. Wang X, Zhang Y, Zhou X, Xia X, Teng W, Sheng L, Ding J. Soy isoflavone reduces LPS-induced acute lung injury via increasing aquaporin 1 and aquaporin 5 in rats. *Open Life Sci* 2023; 18(1): 20220560. <https://doi.org/10.1515/biol-2022-0560>
19. Zhong WJ, Zhang J, Duan JX, Zhang CY, Ma SC, Li YS, Yang NS, Yang HH, Xiong JB, Guan CX, Jiang ZX, You ZJ, Zhou Y. TREM-1 triggers necroptosis of macrophages through mTOR-dependent mitochondrial fission during acute lung injury. *J Transl Med* 2023; 21(1): 179. <https://doi.org/10.1186/s12967-023-04027-4>
20. Han Z, Ma J, Han Y, Yuan G, Jiao R, Meng A. Irisin attenuates acute lung injury by suppressing the pyroptosis of alveolar macrophages. *Int J Mol Med* 2023; 51(4): 32. <https://doi.org/10.3892/ijmm.2023.5235>
21. An HS, Yoo JW, Jeong JH, Heo M, Hwang SH, Jang HM, Jeong EA, Lee J, Shin HJ, Kim KE, Shin MC, Roh GS. Lipocalin-2 promotes acute lung inflammation and oxidative stress by enhancing macrophage iron accumulation. *Int J Biol Sci* 2023; 19(4): 1163-1177. <https://doi.org/10.7150/ijbs.79915>
22. Chen H, Cheng Y, Du H, Zhang C, Zhou Y, Zhao Z, Li Y, Friedemann T, Mei J, Schröder S, Chen M. Shufeng Jiedu capsule ameliorates olfactory dysfunction via the AMPK/mTOR autophagy pathway in a mouse model of allergic rhinitis. *Phyto-medicine* 2022; 107: 154426. <https://doi.org/10.1016/j.phymed.2022.154426>
23. Wu YX, Zeng S, Wan BB, Wang YY, Sun HX, Liu G, Gao ZQ, Chen D, Chen YQ, Lu MD, Pang QF. Sophoricoside attenuates lipopolysaccharide-induced acute lung injury by activating the AMPK/Nrf2 signaling axis. *Int Immunopharmacol* 2021; 90: 107187. <https://doi.org/10.1016/j.intimp.2020.107187>
24. Doolittle LM, Binzel K, Nolan KE, Craig K, Rosas LE, Bernier MC, Joseph LM, Woods PS, Knopp MV, Davis IC. Cytidine 5'-Diphosphocholine Corrects Alveolar Type II Cell Mitochondrial Dysfunction in Influenza-infected Mice. *Am J Respir Cell Mol Biol* 2022; 66(6): 682-693. <https://doi.org/10.1165/rcmb.2021-0512OC>