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Oridonin alleviates inflammation and endoplasmic reticulum stress in pediatric pneumonia via regulating the SIRT1-mediated Wnt/β-catenin signaling pathway

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Summary. Background. Pediatric pneumonia is a prevalent and significant health concern worldwide, with elevated morbidity and mortality rates among affected children. This study was designed to elucidate the therapeutic impact of Oridonin (Ori) on pediatric pneumonia and unravel the underlying mechanisms involved.

Methods. A pediatric infantile pneumonia model was established in mice through intratracheal administration of LPS. Additionally, a cell damage model was created in WI-38 cells by administering LPS. Protein levels were assessed via western blotting, and cell viability was measured with CCK-8. Inflammatory cytokines were quantified through ELISA, and specific assays were employed to evaluate oxidative stress markers. Flow cytometry was utilized to assess cell apoptosis.

Results. Ori alleviated lung inflammation, oxidative stress, apoptosis, and endoplasmic reticulum stress (ERS) in LPS-induced pneumonia mice. In addition, Ori increased the viability of LPS-induced pneumonia cells but decreased cell apoptosis. Furthermore, Ori reduced oxidative stress, inflammation, and ERS in LPS-induced pneumonia cells by enhancing SIRT1 to activate the Wnt/β-catenin pathway.

Conclusion. This study suggested that Ori inhibited pediatric pneumonia by dampening the inflammatory response, oxidative stress, cell apoptosis, and ERS via the SIRT1/Wnt/β-catenin pathway.

Key words: Oridonin, SIRT1, pneumonia, Wnt/β-catenin, inflammation, endoplasmic reticulum stress

Introduction

Pediatric pneumonia poses a significant global health issue, especially impacting children under the age of five, with an annual global death toll of approximately 1.6 million (Muro et al., 2020). Its main clinical symptoms include dyspnea, fever, cough, and breathlessness (Camilloni et al., 2021; Ayan et al., 2022). In the development of pediatric pneumonia, bacterial and viral infections can trigger inflammation in the distal airways of infants, and lead to changes in pulmonary circulation, damage to lung cells, and disruption of normal respiratory functions (Hooven and Polin, 2017). The high prevalence and recurrence of pediatric pneumonia contribute to more severe complications, poorer prognoses, and hindered growth in children, particularly in developing nations (Shah et al., 2017). Consequently, there is a pressing need to delve into the mechanisms of pediatric pneumonia and identify potential therapeutic targets.

Oridonin (Ori), a well-known diterpenoid derived from the Chinese medicinal herb Rabdosia rubescens, exhibits a diverse range of biological properties, including antitumor (Gao et al., 2023), anti-inflammatory (Jia et al., 2019), antioxidant (Zhao et al., 2022), and antibacterial properties (Chen et al., 2023a). Multiple investigations have highlighted the possible role of Ori in the treatment of lung-related ailments. For instance, Ori had inhibitory impacts on myofibroblast differentiation and bleomycin-elicited pulmonary fibrosis by modulating the TGFβ/Smad pathway (Fu et al., 2018). Moreover, Ori demonstrated protective effects against acute lung injury by suppressing the release of IL-1β, IL-6, and TNF-α via the TLR4/MyD88/NF-κB axis (Zhao et al., 2017). Besides, Ori effectively

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Abbreviations. FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDA, Malondialdehyde; SOD, Superoxide dismutase; GSH, Glutathione; TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharide; CCK-8, cell counting kit-8; MPO, Myeloperoxidase; BALF, bronchoalveolar lavage fluid; ELISA, enzyme immunosorbent assay

restrained LPS-elicited early pulmonary fibrosis by repressing NLRP3-dependent inflammation, oxidative stress, and epithelial-mesenchymal transformation (Yang et al., 2022). Nevertheless, conclusive evidence supporting the therapeutic efficacy of Ori in pediatric pneumonia is currently lacking.

Sirtuin 1 (SIRT1) stands out as the extensively studied member within the sirtuin protein family, comprising seven deacetylases that target both histone and nonhistone proteins, requiring NAD (+) as an essential enzymatic cofactor. Studies have highlighted that SIRT1 was implicated in several pathophysiological processes, including anti-inflammation (Wang et al., 2019; Zhang et al., 2020). More importantly, it was reported that Chlorogenic acid activated SIRT1 to suppress HMGB1 acetylation levels and nuclear translocation, thus boosting M2 polarization in alveolar macrophages and relieving Klebsiella pneumoniae-triggered pneumonia (Li et al., 2022). Additionally, evidence supported that SIRT1 regulated the pathogenesis of human cancers or diseases via activating Wnt/β-catenin signaling (Luo et al., 2019; Li et al., 2021). Building upon these discoveries, it is plausible to hypothesize that Ori mitigated pediatric pneumonia via regulating SIRT1-mediated activation of Wnt/β-catenin signaling.

The objective of this study was to explore the potential involvement of Ori in pediatric pneumonia and to assess whether Ori mitigated pulmonary inflammation, oxidative stress, apoptosis, and endoplasmic reticulum stress (ERS) upon LPS through the SIRT1/Wnt/β-catenin pathway. We anticipated that Ori could serve as a promising agent for treating pediatric pneumonia.

Materials and methods

Animal model

Approval for all animal experiment procedures was obtained from the Beijing Friendship Hospital. Male C57BL/6 mice (one week old, weight 4-5 g) were procured from Shanghai SLAC Laboratory Animal Co. Ltd. The mice intraperitoneally received saline or 2 mg LPS/kg for 24h. Random allocation placed mice into four groups, each comprising six animals: control, LPS, LPS+2.5 mg/kg Ori, and LPS+5 mg/kg Ori. The control group mice were instilled with 2 mg/kg LPS dissolved in 50 μL PBS. Ori (2.5 and 5 mg/kg, Sigma) were administered to mice for 1h before LPS treatment. Bronchoalveolar-lavage fluid (BALF) was collected for subsequent ELISA, and lung tissues were gathered for pathological analysis.

Hematoxylin and eosin (H&E) staining

Lung tissues were collected, fixed with formalin, embedded in paraffin, and cut into 5-μm sections. Then, sections were treated with H&E staining for histological evaluation. The pathological changes were evaluated using a light microscope.

Cell culture and treatment

Human embryonic lung fibroblast cells (WI-38) were sourced from ATCC (Rockville, MD, USA) and maintained in MEM supplemented with 10% FBS and 1% Pen/Strep (P/S) at 37°C with 5% CO_2. To establish an *in vitro* model of pediatric pneumonia, WI-38 cells were exposed to 5 μg/ml LPS (Sigma) for 24h at 37°C, following established protocols (Bai et al., 2018; Yu et al., 2022). Then cells were stimulated with increasing doses of Ori (2.5, 5, 10 μM).

Cell transfection

To evaluate the role of SIRT1, negative control si-NC or SIRT1 siRNA (si-SIRT1; a selective inhibitor of SIRT1 RNA, GenePharma Co., Ltd., Shanghai, China) was added to WI-38 cells by Lipofectamine 2000 (Invitrogen) for 48h before LPS administration. The transfection efficiency was verified after transfection at the mRNA and protein level.

CCK-8

Cell viability was assessed utilizing a CCK-8 assay. Briefly, WI-38 cells were seeded in 96-well plates and exposed to 5 μg/ml LPS for 24h at 37°C. Afterward, 10 μl CCK-8 reagent was introduced to each well and the cells were further incubated at 37°C for 2h. Finally, cell viability was quantified by a microplate reader at 450 nm.

Measurement of oxidative stress markers

The cell medium was collected, centrifuged for 10 min at 4°C, and the supernatant was collected. Levels of SOD, GSH, MPO, and MDA were measured using detection kits obtained from Nanjing Jiancheng Bio-Engineering Institute.

ELISA

In brief, the cell medium was collected, centrifuged for 10 min at 4°C, and the supernatant was collected. The concentrations of inflammatory cytokines, including TNF-α (ab181421, Abcam), IL-6 (ab178013, Abcam), and IL-1β (ab229384, Abcam), in the supernatants from WI-38 cells were assessed using ELISA kits.

Flow cytometry

WI-38 cells were trypsinized, washed with ice-cold PBS, and resuspended with binding buffer (Beyotime). Following that, Annexin V-FITC and PI were introduced

to stain cells for 30 min in the dark at 4°C. Flow cytometry (FACSCalibur; BD Biosciences) was employed to detect apoptotic cells.

Western blotting

Cell lysates were prepared using RIPA buffer supplemented with protease and phosphatase inhibitors. Following that, proteins were separated via SDS-PAGE, transferred onto PVDF membranes, and then blocked with skimmed milk. The membranes were subjected to incubation with primary antibodies anti-GRP78 (1:1000, ab108613, Abcam), anti-ATF6 (1:100, ab37149, Abcam), anti-CHOP (1:1000, ab11419, Abcam), anti-Bax (1:1000, ab32503, Abcam), anti-Bcl-2 (1:2000, ab196495, Abcam), anti-Wnt1 (1:1000, ab15251, Abcam), anti-β-catenin (1:400, ab224803, Abcam), anti-cyclin D1 (1:2000, ab134175, Abcam), anti-GAPDH (1:2000, ab9485, Abcam), followed by HRP-conjugated Goat Anti-Rabbit IgG H&L (1:2000, ab6721, Abcam); goat anti-mouse IgG H&L (HRP) (1:10000, ab6789, Abcam). Signal detection was achieved using an ECL detection kit.

Statistical analyses

All data are presented as the mean ± SD and executed using GraphPad Prism 7. Statistical differences were calculated by a Student's t-test or one-way ANOVA. $p<0.05$ was defined as significant. Each experiment was repeated at least three times.

Results

Ori alleviates LPS-induced lung damage

The molecular formula of Ori is presented in Figure 1A. Following induction of infantile pneumonia in mice, we conducted histological examinations of lung tissues. The administration of LPS led to notable tissue damage, characterized by alveolar shrinkage, severe inflammatory cell infiltration, and alveolar wall thickening in pneumonia, while pretreatment with Ori demonstrated a significant reduction in lung damage (Fig. 1B,C). These findings manifested that Ori alleviated lung tissue damage.

Ori inhibits lung inflammation, oxidative stress, and ERS in mice

To unravel the mechanisms underlying Ori's impact on lung injury, inflammatory cytokines were examined. Figure 2A shows that IL-6, IL-1β, and TNF-α levels were enhanced in LPS-induced mice in BALF. However, Ori treatment reversed these changes (Fig. 2A). Moreover, as depicted in Figure 2B, LPS stimulation led to increased levels of MDA and decreased production of SOD and GSH, alterations that were reversed by Ori treatment. Furthermore, western blotting implied that LPS increased the level of ERS marker proteins, including GRP78, ATF6, and CHOP, but Ori diminished levels of GRP78, ATF6, and CHOP (Fig. 2C). In sum, Ori inhibited inflammation, oxidative stress, and ERS in newborn mice.

Ori promotes cell viability and suppresses apoptosis in LPS-induced WI-38 cells

To evaluate the impacts of Ori on LPS-mediated WI-38 cell viability and apoptosis, we conducted CCK-8 and flow cytometry assays. As displayed in Figure 3A,B, LPS treatment hindered viability and promoted apoptosis of WI-38 cells, while administration of Ori promoted WI-38 cell viability and inhibited cell apoptosis in a dose-dependent manner. Meanwhile, western blotting demonstrated that LPS administration diminished Bcl-2 expression and elevated Bax levels, which were reversed by Ori administration (Fig. 3C). These results revealed

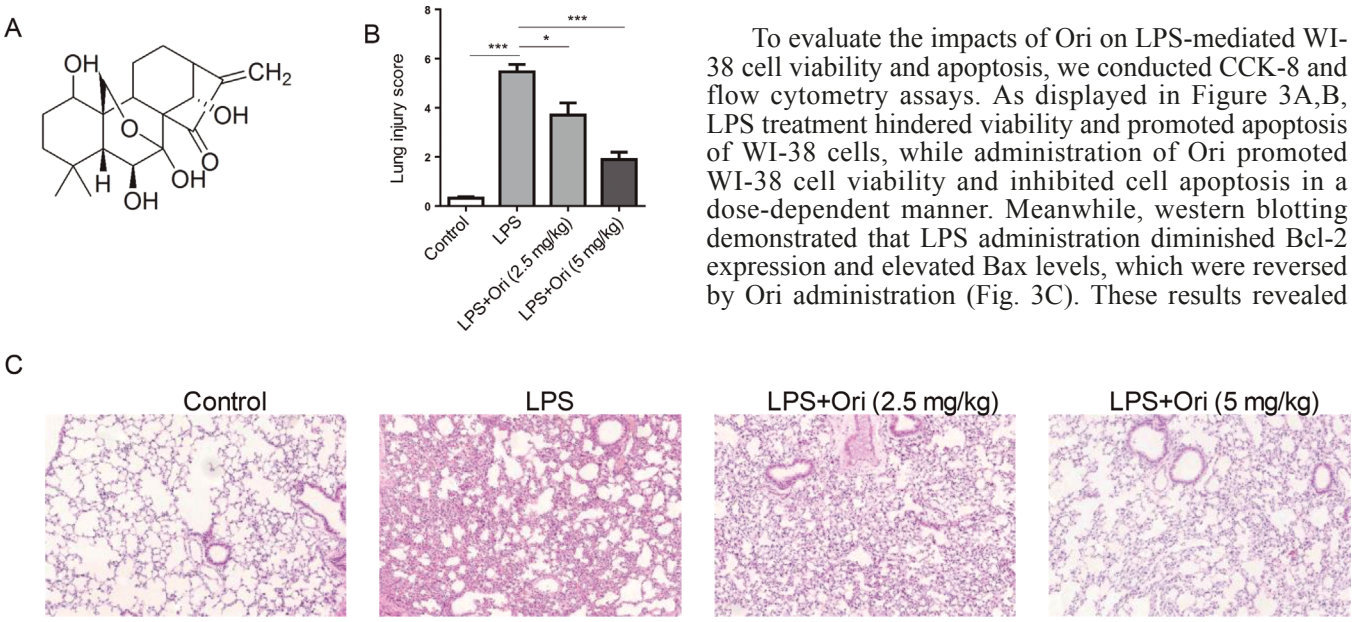

Fig. 1. Ori alleviates LPS-induced lung injury. **A.** The chemical structure of Ori. **B, C.** H&E staining showed the effect of Ori (2.5 and 5 mg/kg) on lung tissue injury in LPS-treated mice and lung injury score.*$p<0.05$; ***$p<0.001$.

that Ori promoted cell viability and decreased apoptosis in LPS-treated WI-38 cells.

Ori mitigates inflammation, oxidative stress, and ERS induced by LPS in WI-38 cells

To assess the impact of Ori on LPS-mediated WI-38 cell inflammation, an ELISA assay was conducted. The results demonstrated an obvious increase in the levels of IL-1β, IL-6, and TNF-α upon LPS stimulation. However, administration of Ori reduced these levels, suggesting that Ori treatment attenuated LPS-mediated WI-38 cell inflammation (Fig. 4A). Next, we found that the expression of MDA was enhanced, whereas that of SOD and GSH declined in LPS-stimulated cells. However, Ori treatment reversed these changes (Fig. 4B). Additionally, we sought to examine whether the protective impact of Ori on LPS-treated WI-38 cells was associated with ERS. Western blotting revealed that LPS treatment substantially elevated the expression of GRP78, ATF6, and CHOP. However, Ori application decreased these protein levels (Fig. 4C). Collectively, these data thus implied that Ori alleviated LPS-triggered cell apoptosis, inflammation, and ERS in WI-38 cells.

Ori activates Wnt/β-catenin signaling by enhancing SIRT1

siRNA against SIRT1 was transfected into WI-38 cells to knockdown SIRT1 expression and the transfection efficiency was confirmed (Fig. 5A). To elucidate whether SIRT1 mitigated LPS-induced WI-38 cell damage through the Wnt/β-catenin pathway, the levels of signaling molecules were examined. The results revealed a dramatic reduction in the levels of Wnt1, β-catenin, and cyclin D1 following LPS stimulation, whereas 10 μM Ori increased these protein levels. Moreover, transfection with si-SIRT1 inhibited Wnt1, β-catenin, and cyclin D1 levels in LPS-induced WI-38 cells. In addition, the Wnt/β-catenin agonist (LiCl)

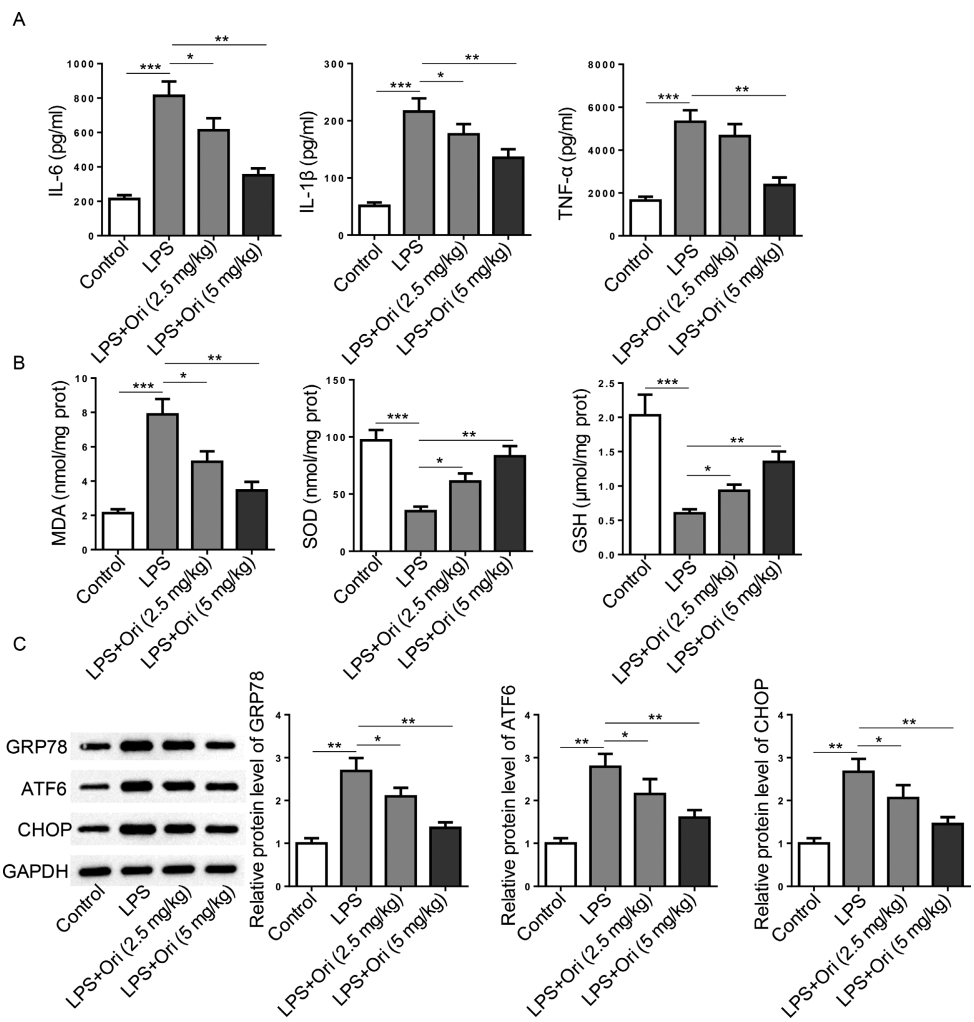

Fig. 2. Ori inhibits lung inflammation, oxidative stress, and ERS in newborn mice. **A.** ELISA showed levels of IL-6, IL-1β, and TNF-α in Control, LPS, LPS+Ori (2.5 mg/kg), and LPS+Ori (5 mg/kg)-treated LPS mice. **B.** The levels of SOD, MDA, and GSH. (C) Western blotting showed the protein levels of GRP78, ATF6, and CHOP in treated mice. The immunoblot signals were quantified by densitometry. *p<0.05; **p<0.01; ***p<0.001.

reversed the inhibitory effect of si-SIRT1 transfection (Fig. 5B,C). Collectively, Ori activated Wnt/β-catenin by elevating SIRT1 expression.

Ori inhibits LPS-induced WI-38 cell injury by regulating SIRT1-mediated Wnt/β-catenin signaling

Finally, the biological significance of SIRT1-mediated Wnt/β-catenin signaling in pediatric pneumonia was evaluated. Figure 6A,B showed that the suppressive effect of Ori on the apoptosis of WI-38 cells under LPS stimulation was abated by SIRT1 deletion, whereas LiCl exhibited similar effects as Ori. Consistently, SIRT1 silencing counteracted the effects of Ori on inflammation, oxidative stress, and ERS marker proteins, whereas LiCl treatment exerted opposite effects with SIRT1 silencing (Fig. 6C-E). Therefore, Ori protected WI-38 cells from LPS-induced damage through the SIRT1/Wnt/β-catenin pathway.

Discussion

Pneumonia is a prevalent, multifaceted, and severe inflammatory lung disease characterized by damage to the alveolar-capillary membrane and the release of inflammatory cytokines due to inflammation, resulting in impaired lung function (Dhanireddy et al., 2006; Nova et al., 2019). Given the urgency of identifying novel therapeutic targets for pneumonia, the role of LPS, a gram-negative bacterial endotoxin, is particularly noteworthy in initiating and significantly contributing to the development of pneumonia-induced acute lung injury (Liu et al., 2022). Consequently, mitigating LPS-triggered lung damage is widely acknowledged as an effective strategy for pneumonia treatment (Chen et al., 2023b; Zhang et al., 2023). In this study, we established a pneumonia mouse model by LPS injection and an *in vitro* model by exposing WI-38 cells to LPS. Multiple evidence has reported Ori as a promising molecular

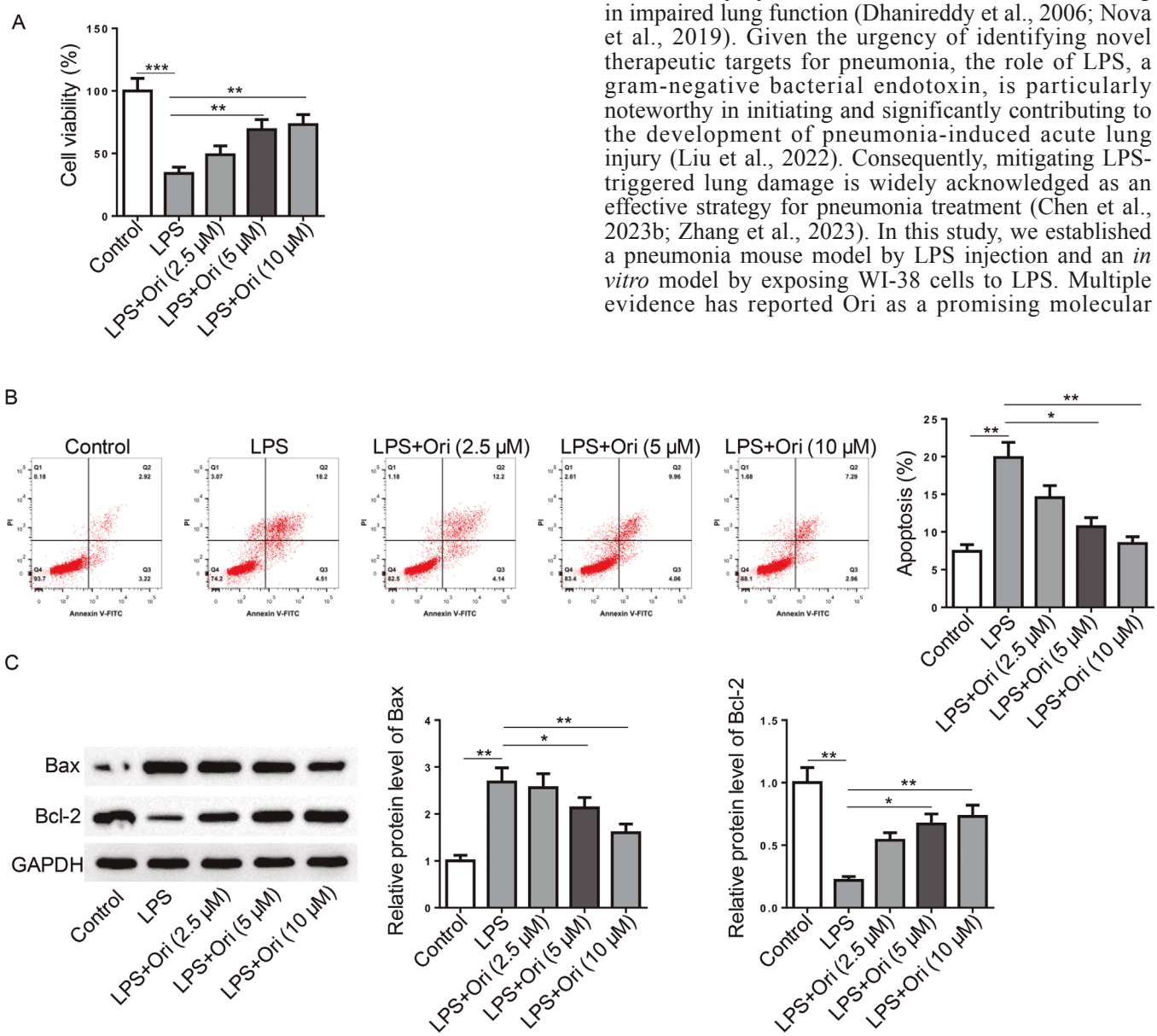

Fig. 3. Ori promotes cell viability and suppresses apoptosis in LPS-induced WI-38 cells. WI-38 cells were assigned into Control, LPS, LPS+Ori (2.5 μM), LPS+Ori (5 μM), and LPS+Ori (10 μM) groups. **A, B.** Cell viability and apoptosis were tested by CCK-8 and flow cytometry assays. **C.** The protein levels of Bax and Bcl-2. The immunoblot signals were quantified by densitometry. *p<0.05; **p<0.01; ***p<0.001.

target in pulmonary diseases via suppressing inflammation (Gao et al., 2022; Yang et al., 2019). Consistent with the aforementioned results, it was found that Ori could relieve pathological damage, and inhibit lung inflammation and oxidative stress in newborn mice subjected to LPS. Moreover, Ori expedited cell viability

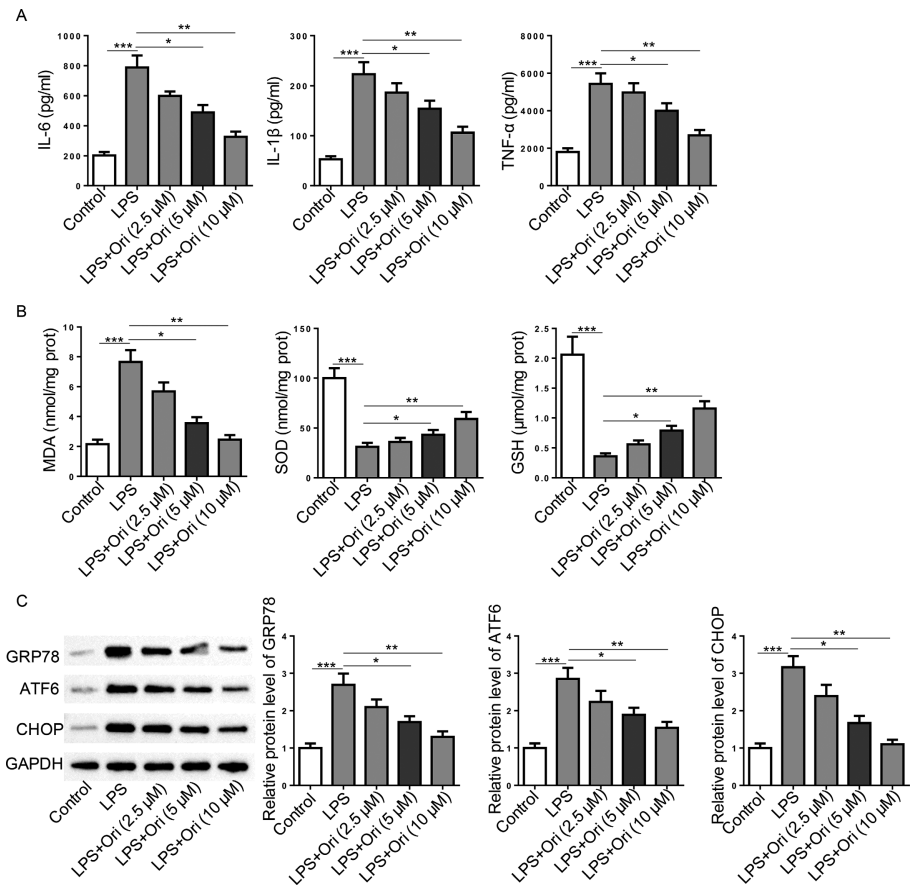

Fig. 4. Ori mitigates LPS-induced inflammation, oxidative stress, and ERS in WI-38 cells. WI-38 cells were assigned into Control, LPS, LPS+Ori (2.5 μM), LPS+Ori (5 μM), and LPS+Ori (10 μM) groups. **A.** ELISA showed levels of IL-6, IL-1β, and TNF-α. **B.** The levels of SOD, MDA, and GSH. **C.** Western blotting showed the protein levels of GRP78, ATF6, and CHOP. The immunoblot signals were quantified by densitometry. *p<0.05; **p<0.01; ***p<0.001.

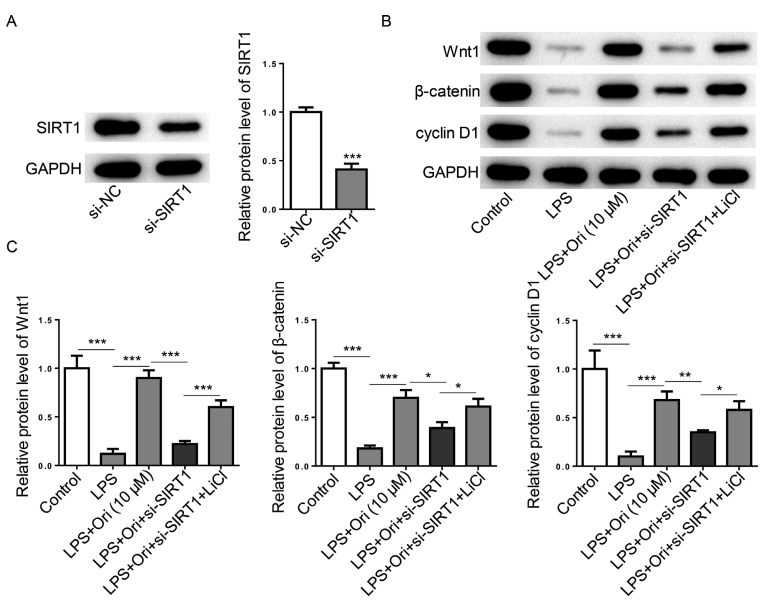

Fig. 5. Ori activates Wnt/β-catenin signaling by upregulating SIRT1. **A.** Western blotting showed si-SIRT1 transfection efficiency at the protein level. **B, C.** Western blotting showed the expression levels of Wnt1, β-catenin, and cyclin D1 in WI-38 cells treated with LPS, LPS+Ori (10 μM), LPS+Ori+si-SIRT1, and LPS+Ori+si-SIRT1+LiCl. The immunoblot signals were quantified by densitometry. *p<0.05; **p<0.01; ***p<0.001.

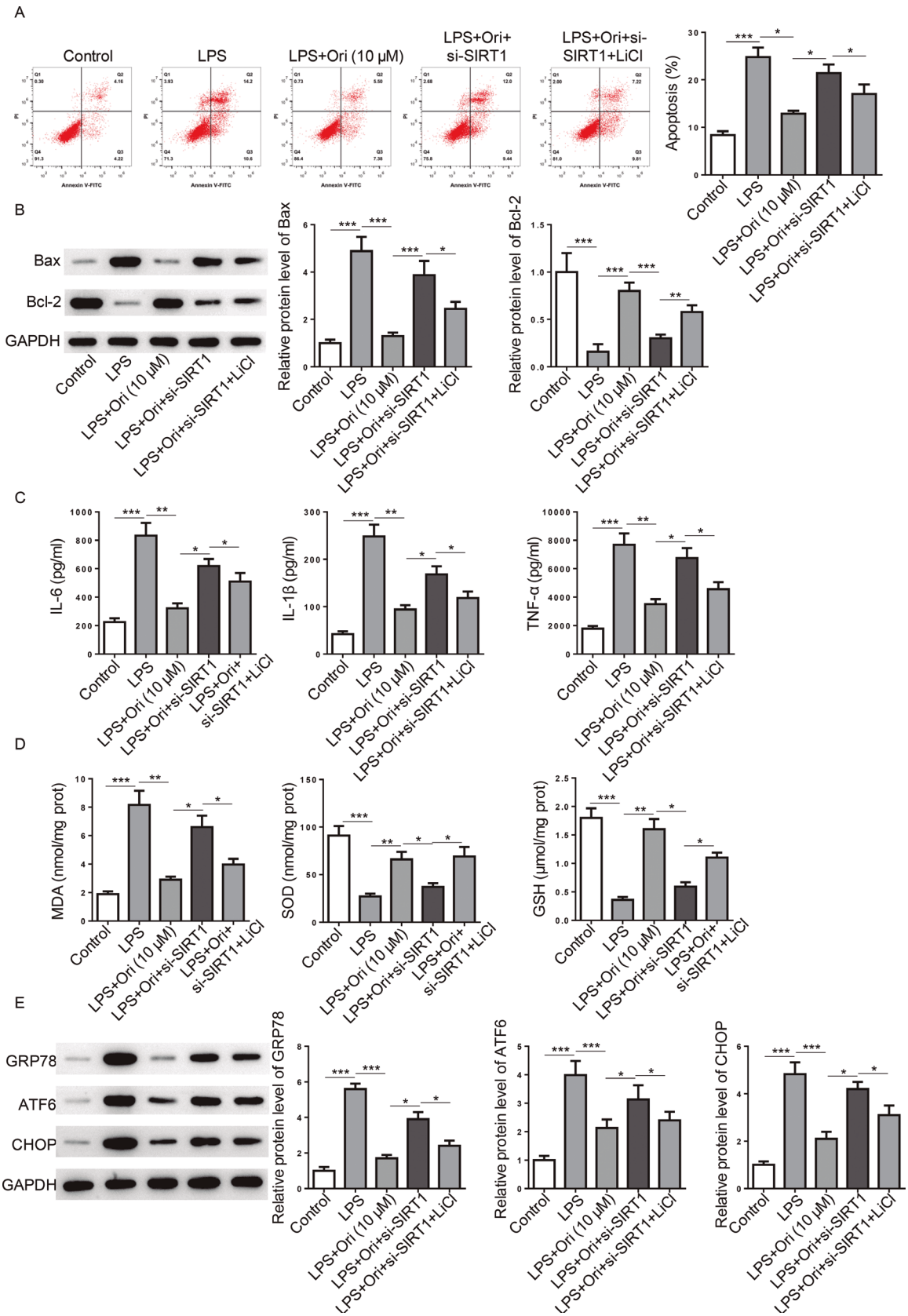

Fig. 6. Ori inhibits LPS-induced WI-38 cell injury by regulating SIRT1-mediated Wnt/β-catenin signaling. WI-38 cells were assigned into Control, LPS, LPS+Ori (10 μM), LPS+Ori+si-SIRT1, and LPS+Ori+si-SIRT1+LiCl. **A, B.** Cell apoptosis was assessed by flow cytometry and western blotting showed the protein levels of Bax and Bcl-2. **C.** ELISA showed levels of IL-6, IL-1β, and TNF-α. **D.** The levels of SOD, MDA, and GSH. **E.** Western blotting showed the protein levels of GRP78, ATF6, and CHOP. The immunoblot signals were quantified by densitometry. *p<0.05; **p<0.01; ***p<0.001.

and hindered apoptosis, inflammation, and oxidative stress in LPS-treated WI-38 cells.

It is widely recognized that ER plays a key function as a regulator of inflammation in various inflammatory diseases, with ERS being recognized as an effective factor in pneumonia. For instance, Xue et al. found that the knockdown of IGF2BP2-regulated STIM1 improved cell damage in the LPS-induced pneumonia cell model by mitigating ERS and the inflammatory response (Xue et al., 2023). Cao et al. reported that alleviating inflammation and ERS in pediatric pneumonia can impede the development of pediatric pneumonia (Cao et al., 2022). Herein, LPS was administered to WI-38 cells to induce ERS injury. The expression of ERS-related proteins, GRP78, ATF6, and CHOP, were increased following LPS induction. However, Ori treatment lowered the levels of ERS-related proteins. These results indicated that Ori inhibited LPS-triggered ERS injury in WI-38 cells.

Growing evidence has suggested that SIRT1 served as a promising molecular target in pulmonary diseases due to its regulatory function in inflammation, oxidative stress, and apoptosis. To cite an instance, Yang et al. showed that depleting miR-146a-3p improved acute lung injury by upregulating SIRT1 and mediating the NF-κB pathway (Yang and Li, 2021). Ding et al. implied that SPHK1 decreased SIRT1 expression to promote mitochondrial permeability transition and increased NLRP3 levels to promote inflammation in a model of infantile pneumonia (Ding et al., 2022). In line with these findings, Ori was found to heighten SIRT1 expression in LPS-stimulated WI-38 cells. The Wnt/β-catenin pathway is a signal transduction cascade activated by the binding of Wnt ligands to membrane protein receptors, and it plays a vital function in various cellular processes, including proliferation, differentiation, ERS, and apoptosis (Nie et al., 2020; Qiu et al., 2021; Chen et al., 2023c). Moreover, the Wnt/β-catenin signaling is known to be implicated in inflammation-associated diseases. For instance, supplementation of FoxM1 protected against LPS-elicited acute lung injury by repressing inflammation and apoptosis by stimulating Wnt/β-catenin signaling (Luo et al., 2023). Herein, we uncovered that Ori elevated the expression of Wnt1, β-catenin, and cyclin D1 through modulating SIRT1, which was consistent with the previous literature, where hyperoside mitigated inflammation, oxidative stress, and apoptosis induced by LPS by enhancing SIRT1 to activate the Wnt/β-catenin and sonic hedgehog pathways (Huang et al., 2021). To further confirm whether SIRT1 also functioned in an LPS-induced *in vitro* model of pneumonia via regulating the Wnt/β-catenin pathway, we introduced LiCl, a Wnt/β-catenin agonist. The results manifested that SIRT1 silencing counteracted the impacts of Ori on LPS-induced WI-38 cell apoptosis, inflammation, oxidative stress, and ERS marker proteins, whereas LiCl treatment exerted opposite effects with SIRT1 deletion.

Conclusion

In summary, our study demonstrated that Ori has a protective impact against pediatric pneumonia by dampening LPS-induced apoptosis, inflammatory response, oxidative stress, and ERS via enhancing SIRT1 expression to activate the Wnt/β-catenin pathway in WI-38 cells. These discoveries confirmed the therapeutic potential of Ori in pediatric pneumonia.

References

Ayan E., Karabulut B. and Ünver H.M. (2022). Diagnosis of pediatric pneumonia with ensemble of deep convolutional neural networks in chest x-ray images. Arab. J. Sci. Eng. 47, 2123-2139.

Bai D., Han A. and Cong S. (2018). The effect of down-regulation of CCL5 on lipopolysaccharide-induced WI-38 fibroblast injury: a potential role for infantile pneumonia. Iran J. Basic Med. Sci. 21, 449-454.

Camilloni A., Nati G., Maggiolini P., Romanelli A., Carbone G., Giannarelli D., Terrenato I., De Marinis M.G., Rossi A., D'Angelo D., Ferrara R., Iacorossi L., Paladini A., Varrassi G., Tarsitani G. and Latina R. (2021). Chronic non-cancer pain in primary care: an Italian cross-sectional study. Signa Vitae 17, 54-62.

Cao X., Wan H. and Wan H. (2022). Urolithin A induces protective autophagy to alleviate inflammation, oxidative stress, and endoplasmic reticulum stress in pediatric pneumonia. Allergol. Immunopathol. 50, 147-153.

Chen G., Yang Z., Wen D., Li P., Xiong Q. and Wu C. (2023a). Oridonin inhibits *Mycobacterium marinum* infection-induced oxidative stress *in vitro* and *in vivo*. 12, 799.

Chen J., Zhao M., Fang W. and Du C. (2023b). Knocking down TNFAIP1 alleviates inflammation and oxidative stress in pediatric pneumonia through PI3K/Akt/Nrf2 pathway. Allergol. Immunopathol. 51, 94-100.

Chen Z., He S., Lian S., Shen Y., Jiang W., Zhou L., Zhou L. and Zhang X. (2023c). The Wnt/β-catenin pathway regulates inflammation and apoptosis in ventilator-induced lung injury. Biosci. Rep. 43, BSR20222429.

Dhanireddy S., Altemeier W.A., Matute-Bello G., O'Mahony D.S., Glenny R.W., Martin T.R. and Liles W.C. (2006). Mechanical ventilation induces inflammation, lung injury, and extra-pulmonary organ dysfunction in experimental pneumonia. Lab. Invest. 86, 790-799.

Ding N., Meng Y., Liu L., Ma S. and Chen Y. (2022). Sphingosine Kinase-1 (SPHK1) promotes inflammation in infantile pneumonia by regulating NLRP3 inflammasome and SIRT1 expression. Histol. Histopathol. 37, 1227-1240.

Fu Y., Zhao P., Xie Z., Wang L. and Chen S. (2018). Oridonin inhibits myofibroblast differentiation and bleomycin-induced pulmonary fibrosis by regulating transforming growth factor β (TGFβ)/Smad pathway. Med. Sci. Monit. 24, 7548-7555.

Gao J., Li C., Wang X., Sun X., Zhang R., Chen C., Yu M., Liu Y., Zhu Y. and Chen J. (2022). Oridonin attenuates lung inflammation and fibrosis in silicosis via covalent targeting iNOS. Biomed. Pharmacother. 153, 113532.

Gao S., Tan H. and Li D. (2023). Oridonin suppresses gastric cancer

SGC-7901 cell proliferation by targeting the TNF-alpha/androgen receptor/TGF-beta signalling pathway axis. J. Cell. Mol. Med. 27, 2661-2674.

Hooven T.A. and Polin R.A. (2017). Pneumonia. Semin. Fetal Neonatal Med. 22, 206-213.

Huang J., Zhou L., Chen J., Chen T., Lei B., Zheng N., Wan X., Xu J. and Wang T. (2021). Hyperoside attenuate inflammation in HT22 cells via upregulating SIRT1 to activities Wnt/β-Catenin and sonic hedgehog pathways. Neural Plast. 2021, 8706400.

Jia T., Cai M., Ma X., Li M., Qiao J. and Chen T. (2019). Oridonin inhibits IL-1β-induced inflammation in human osteoarthritis chondrocytes by activating PPAR-γ. Int. Immunopharmacol. 69, 382-388.

Li Q., Gong Y., Wang Y., Liu B., Chu Y., Gui S., Zheng Y. and Chen X. (2021). Sirt1 promotes the restoration of Hepatic Progenitor Cell (HPC)-mediated liver fatty injury in NAFLD through activating the Wnt/β-Catenin signal pathway. Front. Nutr. 8, 791861.

Li Q.R., Tan S.R., Yang L., He W., Chen L., Shen F.X., Wang Z. and Wang H.F. (2022). Mechanism of chlorogenic acid in alveolar macrophage polarization in Klebsiella pneumoniae-induced pneumonia. J. Leukoc. Biol. 112, 9-21.

Liu Y., Bao C., Deng G. and Ouyang Y. (2022). Arid2-IR downregulates miR-132-3p through methylation to promote LPS-induced ALI in pneumonia. Inhal. Toxicol. 34, 297-303.

Luo Y., Chen J.J., Lv Q., Qin J., Huang Y.Z., Yu M.H. and Zhong M. (2019). Long non-coding RNA NEAT1 promotes colorectal cancer progression by competitively binding miR-34a with SIRT1 and enhancing the Wnt/β-catenin signaling pathway. Cancer Lett. 440-441, 11-22.

Luo Y., Lin S., Mao X., Yang Y., He W., Guo M. and Zeng M. (2023). Overexpression of FoxM1 enhanced the protective effect of bone marrow-derived mesenchymal stem cells on lipopolysaccharide-Induced acute lung injury through the activation of Wnt/β-Catenin signaling. Oxid. Med. Cell. Longev. 2023, 8324504.

Muro R.P., Masoza T.S., Kasanga G., Kayange N. and Kidenya B.R. (2020). Predictors and outcome of first line treatment failure among under-five children with community acquired severe pneumonia at Bugando Medical Centre, Mwanza, Tanzania: A prospective cohort study. PLoS One 15, e0243636.

Nie F., Zhang W., Cui Q., Fu Y., Li H. and Zhang J. (2020). Kaempferol promotes proliferation and osteogenic differentiation of periodontal ligament stem cells via Wnt/β-catenin signaling pathway. Life Sci. 258, 118143.

Nova Z., Skovierova H. and Calkovska A. (2019). Alveolar-capillary membrane-related pulmonary cells as a target in endotoxin-induced acute lung injury. Int. J. Mol. Sci. 20, 831.

Qiu Z., Chen W., Liu Y., Jiang B., Yin L. and Chen X. (2021). LncRNA AC061961.2 overexpression inhibited endoplasmic reticulum stress induced apoptosis in dilated cardiomyopathy rats and cardiomyocytes via activating wnt/β-catenin pathway. J. Recept. Signal Transduc. Res. 41, 494-503.

Shah S.N., Bachur R.G., Simel D.L. and Neuman M.I. (2017). Does this child have pneumonia?: The rational clinical examination systematic review. JAMA 318, 462-471.

Wang Q.L., Yang L., Peng Y., Gao M., Yang M.S. and Xing W. (2019). Ginsenoside Rg1 regulates SIRT1 to ameliorate sepsis-induced lung inflammation and injury via inhibiting endoplasmic reticulum stress and inflammation. Mediators Inflamm. 2019, 6453296.

Xue Z., Li Y., Xiao S., Zhang H. and Xu J. (2023). FOXA2 attenuates lipopolysaccharide-induced pneumonia by inhibiting the inflammatory response, oxidative stress and apoptosis through blocking of p38/STAT3 signaling. Exp. Ther. Med. 26, 469.

Yang Y. and Li L. (2021). Depleting microRNA-146a-3p attenuates lipopolysaccharide-induced acute lung injury via up-regulating SIRT1 and mediating NF-κB pathway. J. Drug Target. 29, 420-429.

Yang H., Lv H., Li H., Ci X. and Peng L. (2019). Oridonin protects LPS-induced acute lung injury by modulating Nrf2-mediated oxidative stress and Nrf2-independent NLRP3 and NF-κB pathways. Cell. Commun. Signal. 17, 62.

Yang H., Wang L., Yang M., Hu J., Zhang E. and Peng L. (2022). Oridonin attenuates LPS-induced early pulmonary fibrosis by regulating impaired autophagy, oxidative stress, inflammation and EMT. Eur. J. Pharmacol. 923, 174931.

Yu Y., Yang T., Ding Z. and Cao Y. (2022). Circ_0026579 alleviates LPS-induced WI-38 cells inflammation injury in infantile pneumonia. Innate Immun. 28, 37-48.

Zhang Y.M., Qu X.Y., Tao L.-N., Zhai J.-H., Gao H., Song Y.-Q. and Zhang S.-X. (2020). XingNaoJing injection ameliorates cerebral ischaemia/reperfusion injury via SIRT1-mediated inflammatory response inhibition. Pharm. Biol. 58, 16-24.

Zhang Q., Yang C., Ma S., Guo S., Hu X., Zhou Z., Liu Y., Zhang X., Jiang R., Zhang Z. and Wen L. (2023). Shiwei Qingwen decoction regulates TLR4/NF-κB signaling pathway and NLRP3 inflammasome to reduce inflammatory response in lipopolysaccharide-induced acute lung injury. J. Ethnopharmacol. 313, 116615.

Zhao G., Zhang T., Ma X., Jiang K., Wu H., Qiu C., Guo M. and Deng G. (2017). Oridonin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-induced RAW264.7 cells and acute lung injury. Oncotarget 8, 68153-68164.

Zhao X.J., Zhu H.Y., Wang X.L., Lu X.W., Pan C.L., Xu L., Pan C.-L., Xu L., Liu X., Xu. and Zhang Z.Y. (2022). Oridonin ameliorates traumatic brain injury-induced neurological damage by improving mitochondrial function and antioxidant capacity and suppressing neuroinflammation through the Nrf2 pathway. J. Neurotrauma. 39, 530-543.

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