Inhibitors of *IFN* Gene Stimulators (*STING*) Improve Intestinal Ischemia-Reperfusion Induced Acute Lung Injury by Activating *AMPK* Signal

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Abstract

Background: Acute lung injury (ALI) caused by intestinal ischemia-reperfusion is a life-threatening disease. Interferon gene stimulator (*STING*) is a cytoplasmic DNA sensor, participating in the initiation of inflammatory response. This study aims to establish whether *C-176* (*STING* inhibitor) improves the ALI under intestinal ischemia-reperfusion conditions.

Methods: To induce ALI, 72 C57BL/6 mice male were subjected to intestinal ischemia-reperfusion for 90 min. Through intraperitoneal injection, *C-176*, a selective *STING* inhibitor was injected 30 minutes before surgical treatment; meanwhile, compound C, an antagonist of adenosine monophosphate-activated protein kinase (*AMPK*) was administered 30 min after surgery. Based on immunofluorescence and Western blot assays, post-ALI assessments included lung water content (TLW), bronchoalveolar lavage fluid (BALF) protein, H&E staining, Masson staining, pulmonary apoptosis (TUNEL, cleaved caspase-3), and pyroptosis (Gasdermin-D [GSDMD], cleaved caspase-1).

Results: *C-176* administration significantly attenuated intestinal ischemia-reperfusion-mediated ALI; this was reflected by exasperated TLW and BALF protein, aggravated lung injury score, elevated degree of pulmonary fibrosis, increased TUNEL- and GSDMD-positive cells, as well as upregulated phosphor-*AMPK*, cleaved caspase-3, and cleaved caspase-1 expression. Moreover, *C-176* increased phosphor-*AMPK* under ALI conditions. Nonetheless, compound C partially reversed these beneficial effects.

Conclusion: *C-176*, a selective *STING* inhibitor improves intestinal ischemia-reperfusion-mediated ALI, and its underlying mechanism may be associated with *AMPK* signal activation.

Introduction

Acute lung injury (ALI) is a severe respiratory disease-causing high global mortality. It is characterized by dyspnea, interstitial edema, accumulation of activated inflammatory cells, mass migration of neutrophils, and diffuse alveolar damage [1, 2]. A growing body of recent evidence indicates that distant lung organs injuries, including abdominal ischemia-reperfusion, infection, and surgery, can cause ALI [3-5]. Moreover, reports indicate that inflammatory response after intestinal ischemia-reperfusion injury promotes ALI [6]. So far, potential treatments or drugs against intestinal ischemia-reperfusion injury-induced ALI are unavailable in clinical therapy.

Interferon gene stimulator (*STING*) is an important mediator of innate immune response; it detects the double-stranded DNA (dsDNA) in the cytoplasm of immune cells including DC cells, T cells, and macrophages [7]. After catalyzing endogenous dsDNA leaking from mitochondria and exogenous dsDNA from pathogens by cyclic *GMP-AMP* synthase (*cGAS*), *STING* translocates from the endoplasmic reticulum to perinuclear microsomes via Golgi apparatus [8]. Notably, *STING* activation takes control of mitochondrial DNA-mediated lung injury by evoking an inflammatory storm [9]. Several studies indicate that lipopolysaccharide (LPS)-induced ALI is associated with upregulation of *STING* expression [10, 11].

So far, limited studies have explored the *STING* function in intestinal ischemia-reperfusion injury-induced ALI.

Although *STING* is essential in the process of ALI, its downstream proteins remain underexplored [12, 13]. Notably, adenosine monophosphate-activated protein kinase (*AMPK*) promotes various anabolic and catabolic signals, hence maintaining suitable levels of adenosine triphosphate under energetic and/or cellular stress [14]. Interestingly, the *STING-AMPK* signal is implicated in high fat diet-induced cardiac anomalies [15]. Moreover, compound C, which is annotated as a reversible inhibitor for *AMPK*, inhibits dsDNA-dependent type I interferon induction [16]. However, the role of the *STING-AMPK* signal in the model of ALI should be further investigated.

This study hypothesizes that a surgical intervention induces intestinal ischemia-reperfusion followed by ALI. Using the selective inhibitor of *STING* and the *AMPK* inhibitor, we aim to the role of *STING-AMPK* signal in the pathogenesis of ALI after intestinal ischemia-reperfusion injury.

Materials And Methods

Adult male C57/BL mice (7–9 weeks old; weight, 27.1±2.3 g) were purchased from Changsheng Biotechnology Co., Ltd. (Benxi, Liaoning, China). All the mice had free access to food and water; they were kept in a 12-h alternating light and dark facility at 25°C ± 1°C (humidity 50%–70%). The tests involving animals were performed following the guidelines of the Animal Ethics Committee of Cangzhou Central Hospital.

Grouping and intestinal ischemia-reperfusion (IR) induced ALI

In the first stage, animals were randomly grouped into five groups: sham (n =24); intestinal ischemia-reperfusion (IR) + vehicle (n =24); IR + C-176 (350 nmol, 4.7 mg/kg) (n =18); IR + C-176 (550 nmol, 7.3 mg/kg) (n =24) and IR + C-176 (750 nmol, 10 mg/kg) (n =18). In the second stage, animals were randomly divided into two groups: IR + C176 + compound C (n =12), and IR + C176 + vehicle (n =12).

Mice were anesthetized with pentobarbital (10 mg/kg) via intraperitoneal injection. An oral endotracheal tube for the mouse was established then connected with a ventilator (Model: volume-controlled; tidal volume: 8 mL/kg; frequency: 120 beats/min). Throughout the experiment, the animals were kept on a warm blanket to maintain the body temperature within 37–38℃. Based on previous studies [17], the IR model was established as follows: (1) a lower midline laparotomy was performed; (2) the superior mesenteric artery was identified and occluded below the celiac trunk with an arterial microclamp, and intestinal ischemia was confirmed by the paleness of the jejunum and ileum; (3) the clamp was removed after 60 min; (4) 0.5 mL of sterile saline at 37℃ was injected into the peritoneal cavity; (5) the incision was sutured and blocked using ropivacaine. At the first stage, a selective *STING* inhibitor, three dosages of C-176 (4.7 mg/kg, 7.3 mg/kg, and 10 mg/kg) (HY-112906, MedChemExpress, NJ, USA) were administered 30 minutes before surgical exposure via intraperitoneal injection. In the second stage, a selective antagonist of *AMPK*, compound C (25 mg/kg) (HY-13418A, MedChemExpress), was

administered via subcutaneous injection 30 min after initiation of IR. Both *C-176* and compound C were dissolved with 10% DMSO (HY-Y0320, MedChemExpress) and 90% corn oil (HY-Y1888, MedChemExpress). Notably, the sham animals underwent a similar procedure without clamps.

Assessment of lung injury

Three hours after IR, mice (n =6) were euthanized through cervical dislocation under 8% sevoflurane. The surface of the left lungs was wiped after rinsing with saline. The weight was recorded as wet weight (W). After drying at 70℃ for 24 h, the dry weight (D) was recorded. Total lung water content (TLW) was calculated using this equation: TLW =(W-D)/D × 100%.

Mice (n =6) were anesthetized under 3–4% sevoflurane and injected with 0.5 mL of normal saline via the trachea. Then, the liquid of bronchoalveolar lavage was gently aspirated and bronchoalveolar lavage was performed twice. After centrifugation with 4000×g at 4 ℃ for 10 min, the supernatant from the lavage solution was obtained as bronchoalveolar lavage fluid (BALF). The content of BALF protein was measured using the BCA assay based on the manufacturers' instructions (P0012, Beyotime, Shanghai, China).

Hematoxylin and eosin (H&E) staining and Masson staining

Three hours after IR, mice (n =6) were anesthetized under 3-4% sevoflurane and perfused with cold saline via ventriculus sinister–aorta. Mice were perfused with 10% paraformaldehyde after the clear saline was released from the right auricle. After fixing with 10% paraformaldehyde for 48 h, left lungs were cut into 5-μm paraffin coronal sections for hematoxylin and eosin (H&E) and Masson staining as previously published [18, 19]. The slides were observed under a light microscope (BX51; Olympus, Tokyo, Japan). Three fields (magnification, ×200) in one slice (3 slices in one group) were randomly selected. Lung injury was analyzed by an experienced investigator blinded to the group, and recorded as normal (0), mild (1), moderate (2), or severe (3), based on histological parameters, including alveolar edema, diffuse alveolar hemorrhage, and congestion, intra-alveolar infiltration of inflammatory cells. Additionally, the percentage of Masson-stained collagen was measured using Image J (1.37v, Wayne Rasband, available through the National Institutes of Health).

Immunofluorescence staining

Slices (thickness: 5-μm) mentioned above were used for immunofluorescence staining. After boiling with sodium citrate at 100℃ for 20 min, the cooled sections were incubated with 1% Triton X-100 for 20 min and blocked with the QuickBlock™ Blocking Buffer (P0260, Beyotime) at 25℃ for 1 h. After washing with PBS three times, sections were overnight incubated with rabbit anti-Gasdermin-D (GSDMD) (K009328P, Solaibio, Beijing, China) at 4 °C. After rising with PBS, the slices were incubated with goat anti-rabbit secondary antibodies (P0208, Beyotime) at room temperature for 1 h, then coated with anti-fluorescence quenching sealing solution with DAPI (P0131, Beyotime) for 5 min and sealed. Terminal deoxynucleotide transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) (C1062, Beyotime) assay was

performed as per the manufacturer's protocol to detect pulmonary apoptosis. Six fields with a magnification of ×200 in 3 slices were randomly selected from each group. Under a fluorescence microscope (BX53, Olympus, Tokyo, Japan), the percentage of GSDMD-positive cells and TUNEL-positive cells were calculated using the Image J (1.37v, Wayne Rasband, available through the National Institutes of Health).

Western blot

After three hours of IR, mice (n =6) were anesthetized under 3–4% sevoflurane and perfused with cold saline via ventriculus sinister–aorta until clear fluid flowed out from the right atrial appendage. Lung tissues were quickly kept on ice. The lung tissues were removed, homogenized on ice; the total protein was extracted and quantified through the BCA assay. Each sample containing 40 μg of mixed with loading buffer was boiled at 100℃ for 15 minutes. The sample was separated with 10% SDS-PAGE and transferred to a PVDF membrane. The membrane was incubated with QuickBlock™ blocking buffer (P0235, Beyotime) at 25℃ for 10 min, then rinsed with Western wash buffer (P0023C, Beyotime) for 5 min to 3 times. Anti-rabbit *p-AMPK* (dilution: 1:500, ab133448, Abcam, Cambridge, UK), anti-rabbit AMPK (dilution: 1:1000, ab32047, Abcam, Cambridge, UK), anti-rabbit cleaved-caspase-1 (dilution: 1:500, ab179515, Abcam, Cambridge, UK), anti-rabbit cleaved-caspase-3 (dilution: 1:500, ab32351, Abcam, Cambridge, UK) and GADPH (dilution 1:1000, K106389P, Solaibio) were used for overnight incubation of PVDF membranes at 4 ℃. After rising with Western washing buffer 3 times, the membranes were incubated with goat anti-rabbit secondary antibody (dilution 1:1000, A0562, Beyotime) at 25 ℃ for 1 h. After washing 3 times with Western washing buffer, protein bands were detected using the BeyoECL Moon (P0018, Beyotime). The ratio between the gray value of the target protein to GAPDH bands (internal reference) was calculated using Image J.

Statistical analysis

All results were expressed as the Means ± standard deviation (SD). Statistical analysis was performed using student's t-test or one-way analysis of variance (ANOVA) for multiple comparisons. Differenceswith *P* values of less than 0.05 (P<0.05) were considered statistically significant.

Results

C-176 mitigates lung injury after intestinal ischemia-reperfusion injury

C-176 (a selective inhibitor of *STING*) was introduced to explore the potential role of *STING* in intestinal ischemia-reperfusion-induced ALI. Both TLW and BALF protein was used to measure pulmonary edema and exudation. Besides, H&E and Masson staining were used to evaluate pulmonary structure damage and fibrosis, respectively. In contrast with sham group, both TLW ($F_{4, 25}$ =215.9, *P* <0.0001; Fig. 2A) and BALF protein ($F_{4, 25}$ =523.3, *P* <0.0001; Fig. 2B) were significantly increased in the IR + vehicle group. Similarly, lung injury score ($F_{4, 25}$ =195.7, *P* <0.0001; Fig. 2C, D) indicated by H&E staining and the

percentage of collagen volume fraction ($F_{4, 25}$ =278.8, *P* <0.0001; Fig. 2E, F) revealed by Masson staining were increased in the IR + vehicle group. Nonetheless, TLW, BALF protein, pathological score, and the percentage of collagen volume fraction were significantly decreased with increasing dose of *C-176* (Fig. 2A–F). No remarkable difference of index mentioned above was noted between the mice exposed to intestinal ischemia-reperfusion injury with 550 nmol and 750 nmol. Thus, 550 nmol of C-176 was applied in the follow-up study.

C-176 mitigates pulmonary apoptosis and pyroptosis induced by intestinal ischemia-reperfusion injury

Immunofluorescence assays for TUNEL (apoptosis) and GDSMD (pyroptosis) were used to evaluate the effects of *STING* on pulmonary apoptosis and pyroptosis induced by intestinal ischemia-reperfusion injury. During the late stages of apoptosis, DNA degradation in the nuclei could be detected by the TUNEL assay [20]. As a pyroptosis execution protein, GSDMD is usually used to explore the classical pyroptosis activated by cleaved caspase-1[21]. Immunofluorescence showed an increased number of TUNEL-positive ($F_{2, 15}$ =217.1, *P* <0.0001; Fig. 3A, B) and GSDMD-positive ($F_{2, 15}$ =162.4, *P* <0.0001; Fig. 3C, D) cells in the IR + Vehicle group compared to that in the sham group. Nonetheless, unlike the IR + Vehicle group, both TUNEL ($F_{2, 15}$ =162.4, *P* <0.0001; Fig. 3A, B) and GSDMD-positive cells ($F_{2, 15}$ =217.1, *P* <0.0001; Fig. 3C, D) were significantly decreased in the IR + C-176 group. Additionally, Western blot results showed that the expressions of apoptosis-associated factors cleaved-caspase-3 ($F_{2, 15}$ =1082, *P* <0.0001; Fig. 4A, B) and pyroptosis-associated factors cleaved-caspase-1 ($F_{2, 15}$ =148.3, *P* <0.0001; Fig. 4A, C) were significantly upregulated in the IR + Vehicle group compared to that in the sham group; on the other hand, *C-176* partially reversed this upregulation in the IR + C-176 group ($F_{2, 15}$ =1082, *P* <0.0001 for cleaved caspase-3; $F_{2 ,15}$ =148.3, *P* <0.0001 for cleaved caspase-1; Fig. 4A–C). The above results indicate that the *STING* inhibitor *C-176* mitigates apoptosis and pyroptosis in the lung after intestinal ischemia-reperfusion injury.

AMPK signal is involved in the protective effects of *C-176* against lung injury induced by intestinal ischemia-reperfusion injury

AMPK signal is a downstream factor of *STING* in several processes of inflammatory injury [22, 23]. The ratio of phosphor-*AMPK* to total *AMPK* was slightly increased in mice exposed to IR compared to those under sham treatment (IR + Vehicle vs. sham, $F_{2, 15}$ =348.6, *P* <0.0001; Fig. 5A, B). Interestingly, the ratio of phosphor-*AMPK* to total *AMPK* was further upregulated in mice exposed to IR plus *C-176* treatment compared to those exposed to IR plus vehicle (IR + C-176 vs. IR + Vehicle, $F_{2, 15}$ =348.6, *P* <0.0001; Fig. 5A, B). These findings show that the *AMPK* signal may regulate the protective effects of *C-176* against lung injury induced by IR.

Compound C, an *AMPK* inhibitor, could reverse the protective effect of *C-176* on ALI

Compound C (a selective inhibitor of AMPK) was introduced to further detect the role of *STING-AMPK* signal in the lung injury induced by intestinal ischemia-reperfusion injury. Based on Western blot results,

compound C significantly decreased the ratio of phosphor-*AMPK*/total *AMPK* in the IR + *C-176* + compound C group relative to IR + *C-176* + Vehicle group (t =11.52, *P* <0.0001; Fig. 6A, B). Unlike the IR + *C-176* + Vehicle group, mice in the IR + *C-176* + compound C group showed a significant increase TLW (t =10.85, *P* <0.0001; Fig. 7A); an elevation of BALF protein content (t =11.86, *P* <0.0001; Fig. 6B); an aggravation of lung injury score (t =6.167, *P* <0.001; Fig. 7C, D); and an upregulation in the percentage of collagen volume (t =6.005, *P* <0.001; Fig. 7E, F). Additionally, TUNEL- and GSDMD-positive cells in the lung were both increased in the IR + *C-176* + compound C group relative to IR + *C-176* + Vehicle group (t =5.478, *P* <0.001 for TUNEL; t =3.543, *P* <0.01 for GSDMD; Fig. 8A–D). Moreover, both expressions of apoptosis-associated factors cleaved caspase-3 (t =6.078, *P* <0.001; Fig. 8E, F) and pyroptosis-associated factors cleaved caspase-1 (t =8.959, *P* <0.001; Fig. 8E, G) were significantly upregulated in the IR +Vehicle group compared to that in the sham group.

Discussion

The present report details a *STING– AMPK* signal in the pathological process of ALI after intestinal ischemia-reperfusion injury. The main findings included: (1) the *STING C-176* inhibitor significantly reduced lung injury and pulmonary fibrosis after intestinal ischemia-reperfusion injury; (2) *STING C-176* inhibitor significantly ameliorated pulmonary apoptosis and pyroptosis; (3) the protective effects of *C-176* against ALI after intestinal ischemia-reperfusion injury may be associated with *STING– AMPK* signal.

Endogenous toxins released from intestinal bacteria can shift to the circulatory system after intestinal ischemia-reperfusion injury, resulting in systemic inflammation, including lung injury [24]. Besides endogenous toxins, inflammatory factors including *IL-1* and *IL-6* produced during ischemia enter the circulatory system [25]. Relevant studies revealed that distant lung injury occurs during the process of reperfusion after intestinal ischemia; however, the mechanism of lung injury after intestinal ischemia-reperfusion injury remains unclear [26, 27]. Data showed that lung injury indicated by TLW, the content of BALF protein, pathological score, and collagen volume fraction was significantly aggravated after intestinal ischemia-reperfusion injury. This implies that this rodent model of intestinal ischemia-reperfusion injury potentially triggers distant lung injury, which can destroy the alveolar membrane.

The formation and activation of the inflammasome are facilitated by the *cGAS-STING* pathway, subsequently causing pyroptosis and apoptosis [28, 29]. Recent studies have reported that inflammasome response after bacterial and viral infections is ameliorated by inhibiting the *cGAS-STING-NLRP3* axis in the human myeloid cells [10]. Benmerzoug et. al reported that *STING*-mediated self-dsDNA sensing regulates the process of silica-induced lung inflammation [30]. The *cGAS-STING-NLRP3* axis in the cytoplasm is a potential therapeutic target against ALI [31]. Interestingly, *STING* inhibition through a selective inhibitor (*C-176*) significantly attenuated pulmonary inflammation and fibrosis in mice induced by graphitized multi-walled carbon nanotubes [32]. We showed that *C-176* effectively reduced pulmonary pyroptosis and apoptosis caused by intestinal ischemia-reperfusion injury, and mitigated ALI. Considering the role of *C-176*, our results confirm the activation of the *STING* signal in ALI after intestinal ischemia-reperfusion injury.

A recent publication reported that the *STING-TBK1* complex inhibits phosphorylation of *AMPK*, thereby enhancing inflammatory response in *vivo* and *vitro* [33]. Additionally, *AMPK/SIRT1* activation protects ALI induced by LPS by inhibiting pulmonary apoptosis indicated by cleaved caspase-3 reduction [34]. Endotoxin-induced ALI correlates with pyroptosis via the *AMPK/NLRC4* pathways [35]. We found a slight increase of *AMPK* phosphorylation in pulmonary tissue after intestinal ischemia-reperfusion injury; however, *C-176* could further increase phosphor-*AMPK*. Intriguingly, compound C, an inhibitor of *AMPK* significantly decreased phosphor-*AMPK*, partially eliminating the protective effects of *C-176*. These data indicate that the *STING-AMPK* signal is implicated in the process of ALI after intestinal ischemia-reperfusion injury.

In conclusion, we evaluated the role of the *STING-AMPK* signal in the pathophysiological process after ALI induced by intestinal ischemia-reperfusion injury. The findings support the fact that the *STING-AMPK* signal is a potentially novel therapeutic approach for the treatment of intestinal ischemia-reperfusion injury-induced ALI.

Declarations

Data Availability Statement

All datasets generated for this study are included in the article

Consent for publication

Not Applicable

Acknowledgements

Not applicable.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal testing was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Review Board of Cangzhou Central Hospital.

Competing interests

The authors have no conflicts of interest to declare.

Authors' contributions

M.Y. and J.T.N. performed the study and drafted the manuscript;

Y.X.M., H.B.W., L.Z. and Y.Z. helped to conduct the design of study and acquire the data;

M.Y. and P.S.W. performed the statistical analyses;

J.T.N. conceived the study and revised the manuscript;

All authors have read and approved the final manuscript.

References

1. Butt Y, Kurdowska A, Allen TC: **Acute Lung Injury: A Clinical and Molecular Review**. *Arch Pathol Lab Med* 2016, **140**(4):345-350.
2. Hughes KT, Beasley MB: **Pulmonary Manifestations of Acute Lung Injury: More Than Just Diffuse Alveolar Damage**. *Arch Pathol Lab Med* 2017, **141**(7):916-922.
3. Li Y, Cao Y, Xiao J, Shang J, Tan Q, Ping F, Huang W, Wu F, Zhang H, Zhang X: **Inhibitor of apoptosis-stimulating protein of p53 inhibits ferroptosis and alleviates intestinal ischemia/reperfusion-induced acute lung injury**. *Cell Death Differ* 2020, **27**(9):2635-2650.
4. Islam D, Huang Y, Fanelli V, Delsedime L, Wu S, Khang J, Han B, Grassi A, Li M, Xu Y *et al*: **Identification and Modulation of Microenvironment Is Crucial for Effective Mesenchymal Stromal Cell Therapy in Acute Lung Injury**. *Am J Respir Crit Care Med* 2019, **199**(10):1214-1224.
5. Deshpande R, Zou C: **Pseudomonas Aeruginosa Induced Cell Death in Acute Lung Injury and Acute Respiratory Distress Syndrome**. *Int J Mol Sci* 2020, **21**(15).
6. Tan Y, Zuo W, Huang L, Zhou B, Liang H, Zheng S, Jia W, Chen S, Liu J, Yang X *et al*: **Nervilifordin F alleviates intestinal ischemia/reperfusion-induced acute lung injury via inhibiting inflammasome and mTOR pathway**. *Int Immunopharmacol* 2020, **89**(Pt A):107014.
7. Wu J, Dobbs N, Yang K, Yan N: **Interferon-Independent Activities of Mammalian STING Mediate Antiviral Response and Tumor Immune Evasion**. *Immunity* 2020, **53**(1):115-126.e115.
8. Haag SM, Gulen MF, Reymond L, Gibelin A, Abrami L, Decout A, Heymann M, van der Goot FG, Turcatti G, Behrendt R *et al*: **Targeting STING with covalent small-molecule inhibitors**. *Nature* 2018, **559**(7713):269-273.
9. Liu Q, Wu J, Zhang X, Li X, Wu X, Zhao Y, Ren J: **Circulating mitochondrial DNA-triggered autophagy dysfunction via STING underlies sepsis-related acute lung injury**. *Cell Death Dis* 2021, **12**(7):673.

10. Ning L, Wei W, Wenyang J, Rui X, Qing G: **Cytosolic DNA-STING-NLRP3 axis is involved in murine acute lung injury induced by lipopolysaccharide**. *Clin Transl Med* 2020, **10**(7):e228.
11. Comish PB, Liu MM, Huebinger R, Carlson D, Kang R, Tang D: **The cGAS-STING pathway connects mitochondrial damage to inflammation in burn-induced acute lung injury in rat**. *Burns* 2021.
12. Balka KR, Louis C, Saunders TL, Smith AM, Calleja DJ, D'Silva DB, Moghaddas F, Tailler M, Lawlor KE, Zhan Y *et al*: **TBK1 and IKKε Act Redundantly to Mediate STING-Induced NF-κB Responses in Myeloid Cells**. *Cell Rep* 2020, **31**(1):107492.
13. Balka KR, De Nardo D: **Molecular and spatial mechanisms governing STING signalling**. *Febs j* 2021, **288**(19):5504-5529.
14. Zhu JK: **Abiotic Stress Signaling and Responses in Plants**. *Cell* 2016, **167**(2):313-324.
15. Gong Y, Li G, Tao J, Wu NN, Kandadi MR, Bi Y, Wang S, Pei Z, Ren J: **Double knockout of Akt2 and AMPK accentuates high fat diet-induced cardiac anomalies through a cGAS-STING-mediated mechanism**. *Biochim Biophys Acta Mol Basis Dis* 2020, **1866**(10):165855.
16. Peng Y, Zhuang J, Ying G, Zeng H, Zhou H, Cao Y, Chen H, Xu C, Fu X, Xu H *et al*: **Stimulator of IFN genes mediates neuroinflammatory injury by suppressing AMPK signal in experimental subarachnoid hemorrhage**. *J Neuroinflammation* 2020, **17**(1):165.
17. Kim JH, Kim J, Chun J, Lee C, Im JP, Kim JS: **Role of iRhom2 in intestinal ischemia-reperfusion-mediated acute lung injury**. *Sci Rep* 2018, **8**(1):3797.
18. Tang J, Xu L, Zeng Y, Gong F: **Effect of gut microbiota on LPS-induced acute lung injury by regulating the TLR4/NF-kB signaling pathway**. *Int Immunopharmacol* 2021, **91**:107272.
19. Song C, He L, Zhang J, Ma H, Yuan X, Hu G, Tao L, Zhang J, Meng J: **Fluorofenidone attenuates pulmonary inflammation and fibrosis via inhibiting the activation of NALP3 inflammasome and IL-1β/IL-1R1/MyD88/NF-κB pathway**. *J Cell Mol Med* 2016, **20**(11):2064-2077.
20. Kyrylkova K, Kyryachenko S, Leid M, Kioussi C: **Detection of apoptosis by TUNEL assay**. *Methods Mol Biol* 2012, **887**:41-47.
21. Karmakar M, Minns M, Greenberg EN, Diaz-Aponte J, Pestonjamasp K, Johnson JL, Rathkey JK, Abbott DW, Wang K, Shao F *et al*: **N-GSDMD trafficking to neutrophil organelles facilitates IL-1β release independently of plasma membrane pores and pyroptosis**. *Nat Commun* 2020, **11**(1):2212.
22. Peng Y, Zhuang J, Ying G, Zeng H, Zhou H, Cao Y, Chen H, Xu C, Fu X, Xu H: **Stimulator of IFN genes mediates neuroinflammatory injury by suppressing AMPK signal in experimental subarachnoid hemorrhage**. *Journal of Neuroinflammation* 2020, **17**.
23. Prantner D, Perkins DJ, Vogel SN: **AMP-activated Kinase (AMPK) Promotes Innate Immunity and Antiviral Defense through Modulation of Stimulator of Interferon Genes (STING) Signaling**. *Journal of Biological Chemistry* 2016:292.
24. Qian J, Li G, Jin X, Ma C, Cai W, Jiang N, Zheng J: **Emodin protects against intestinal and lung injury induced by acute intestinal injury by modulating SP-A and TLR4/NF-κB pathway**. *Biosci Rep* 2020, **40**(9).

25. Yuan B, Xiong LL, Wen MD, Zhang P, Ma HY, Wang TH, Zhang YH: **Interleukin-6 RNA knockdown ameliorates acute lung injury induced by intestinal ischemia reperfusion in rats by upregulating interleukin-10 expression**. *Mol Med Rep* 2017, **16**(3):2529-2537.
26. Wu D, Wang J, Li H, Xue M, Ji A, Li Y: **Role of Hydrogen Sulfide in Ischemia-Reperfusion Injury**. *Oxid Med Cell Longev* 2015, **2015**:186908.
27. Jin C, Chen J, Gu J, Zhang W: **Gut-lymph-lung pathway mediates sepsis-induced acute lung injury**. *Chin Med J (Engl)* 2020, **133**(18):2212-2218.
28. Gaidt MM, Ebert TS, Chauhan D, Ramshorn K, Pinci F, Zuber S, O'Duill F, Schmid-Burgk JL, Hoss F, Buhmann R *et al*: **The DNA Inflammasome in Human Myeloid Cells Is Initiated by a STING-Cell Death Program Upstream of NLRP3**. *Cell* 2017, **171**(5):1110-1124.e1118.
29. McArthur K, Whitehead LW, Heddleston JM, Li L, Padman BS, Oorschot V, Geoghegan ND, Chappaz S, Davidson S, San Chin H *et al*: **BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis**. *Science* 2018, **359**(6378).
30. Benmerzoug S, Rose S, Bounab B, Gosset D, Duneau L, Chenuet P, Mollet L, Le Bert M, Lambers C, Geleff S *et al*: **STING-dependent sensing of self-DNA drives silica-induced lung inflammation**. *Nat Commun* 2018, **9**(1):5226.
31. Wang W, Hu D, Wu C, Feng Y, Li A, Liu W, Wang Y, Chen K, Tian M, Xiao F *et al*: **STING promotes NLRP3 localization in ER and facilitates NLRP3 deubiquitination to activate the inflammasome upon HSV-1 infection**. *PLoS Pathog* 2020, **16**(3):e1008335.
32. Han B, Wang X, Wu P, Jiang H, Yang Q, Li S, Li J, Zhang Z: **Pulmonary inflammatory and fibrogenic response induced by graphitized multi-walled carbon nanotube involved in cGAS-STING signaling pathway**. *J Hazard Mater* 2021, **417**:125984.
33. Konno H, Konno K, Barber GN: **Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling**. *Cell* 2013, **155**(3):688-698.
34. Li X, Jamal M, Guo P, Jin Z, Zheng F, Song X, Zhan J, Wu H: **Irisin alleviates pulmonary epithelial barrier dysfunction in sepsis-induced acute lung injury via activation of AMPK/SIRT1 pathways**. *Biomed Pharmacother* 2019, **118**:109363.
35. He Y, Xu K, Wang Y, Chao X, Xu B, Wu J, Shen J, Ren W, Hu Y: **AMPK as a potential pharmacological target for alleviating LPS-induced acute lung injury partly via NLRC4 inflammasome pathway inhibition**. *Exp Gerontol* 2019, **125**:110661.

Figures

Figure 1

Experimental schematic diagram

Mice with intestinal ischemia-reperfusion injury (IR) and *C-176* treatment. IR: Mice received an occlusion of the superior mesenteric artery below the celiac trunk with an arterial microclamp for 2 h. Sham: Mice subjected to identical surgery except for the occlusion of the superior mesenteric artery. *C-176*: *C-176* administered via intraperitoneal injection 30 min before surgical exposure. Compound C: Compound C administered via subcutaneous injection 30 min after initiation of IR. Vehicle: 10% DMSO and 90% corn oil were administered via intraperitoneal or subcutaneous injection as a control. Sham: Mice subjected to identical surgery and vehicle injection except for the occlusion of the superior mesenteric artery.

Figure 2

Pathological effects of *STING* on lung injury induced by IR

(A) Total lung water content (TLW); and (B) content of BALF protein caused by the indicated stimuli 3 h after IR; (C) Representative photomicrographs of H&E; and (E) Masson trichrome-stained sections. Scale bar =50 μm; (D) Lung injury score; and (F) the percentage of lung collagen content caused by the indicated stimuli 3 h after IR. Data are presented as the mean ± SD (n =6). Sham, IR, C-176, and vehicle are described above. ****P <0.0001.

Figure 3

C-176 mitigates pulmonary apoptosis and pyroptosis in mice exposed to IR

(A) Representative photomicrographs of TUNEL-positive cells; and (C) GSDMD-positive cells in the lung; (B) The percentage of TUNEL-positive cells; and (D) GSDMD-positive cells in the lung caused by the indicated stimuli 3 h after IR. Data are presented as the mean ± SD (n =6). Sham, IR, *C-176*, and vehicle are described above. ****P <0.0001, ***P <0.001, **P <0.01.

Figure 4

C-176 mitigates pulmonary apoptosis-associated and pyroptosis-associated factors in mice exposed to IR

(A) Representative Western blot of cleaved caspase-3 and cleaved caspase-1 in the lung caused by the indicated stimuli; (B) The ratio between the optical density value of cleaved caspase-3 and GAPDH in the lung, as evaluated by Western blot; (C) The ratio between the optical density value of cleaved caspase-3 and GAPDH in the lung, as evaluated by Western blot. Sham, IR, *C-176*, and vehicle are described above. ****P <0.0001.

Figure 5

C-176 upregulated phosphor-*AMPK* expression in mice exposed to IR

(A) Representative Western blot of phosphor-*AMPK* and total *AMPK* in the lung caused by the indicated stimuli; (B) The ratio between the optical density value of phosphor-*AMPK* and total *AMPK* in the lung, as evaluated by Western blot. Sham, IR, *C-176*, and vehicle are described above. ****P <0.0001.

Figure 6

Compound C reverses phosphor-*AMPK* expression in mice exposed to IR

(A) Representative Western blot of phosphor-*AMPK* and total *AMPK* in the lung caused by the indicated stimuli; (B) The ratio between the optical density value of phosphor-*AMPK* and total *AMPK* in the lung, as evaluated by Western blot. IR, *C-176*, compound C and vehicle are described above. ****P <0.0001.

Figure 7

Compound C reverses the protective effects against ALI in mice exposed to IR (A) Total lung water content (TLW); and (B) content of BALF protein caused by the indicated stimuli 3 h after IR; (C) Representative photomicrographs of H&E; and (E) Masson trichrome-stained sections. Scale bar =50 μm; (D) Lung injury score; and (F) the percentage of lung collagen content caused by the indicated stimuli 3 h after IR. Data are presented as the mean ± SD (n =6). IR, C-176, compound C and vehicle are described above. ****P <0.0001, ***P <0.001.

Figure 8

Compound C reverses the anti-apoptotic and anti-pyroptotic effects of *C-176* against ALI in mice exposed to IR

(A) Representative photomicrographs of TUNEL-positive cells; and (C) GSDMD-positive cells in the lung; (B) The percentage of TUNEL-positive cells; and (D) GSDMD-positive cells in the lung caused by the indicated stimuli 3 h after IR; (E) Representative Western blot of cleaved caspase-3 and cleaved caspase-1 in the lung caused by the indicated stimuli; (F) The ratio between the optical density value of cleaved

caspase-3 and GAPDH in the lung, as evaluated by Western blot; (G) The ratio between the optical density value of cleaved caspase-3 and GAPDH in the lung, as evaluated by Western blot. Data are presented as the mean ± SD (n =6). Sham, IR, *C-176*, and vehicle are described above. ***P <0.001, **P <0.01.