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Angptl2 deficiency attenuates paraquat (PQ)-induced lung injury in mice by altering inflammation, oxidative stress and fibrosis through NF-κB pathway

Wei Yang [a, 1], Wen Liu [a, b, 1], Wei Yu [a, 1], Dongsheng Fei [a], Xianglin Meng [a], Songlin Yang [a], Shishuai Meng [a], Mingyan Zhao [a, *]

[a] Department of Critical Care Medicine, The First Affiliated Hospital of Harbin Medical University, No. 23, YouZheng Street, Nangang District, Harbin, 150001, China

[b] Department of General Surgery, Xinxiang Medical University, No. 601, New Yan Road, Xinxiang 453000, China

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ABSTRACT

Paraquat (PQ) is one of the most extensively used herbicides, possessing high toxicity for humans and animals. The lung is the main target organ by the poisoning of PQ resulting in acute lung injury. Nonetheless, molecular mechanisms underlying PQ-induced lung injury remain unclear. Here, we ask if angiopoietin-like protein 2 (Angptl2), a pro-inflammatory protein, contributes to inflammation that accelerates acute lung injury. The results indicated that abundant Angptl2 expression was observed in lung tissues of PQ-treated mice. Histological analysis revealed that PQ-induced histological changes were alleviated by Angptl2 knockout (Angptl2$^{-/-}$). Angptl2$^{-/-}$ in PQ-treated mice attenuated acute lung injury progression by reducing the number of total cells, total leukocytes, neutrophils and macrophages in bronchoalveolar lavage fluid (BALF) and reducing inflammatory response through the inactivation of nuclear factor kappa B (NF-κB) pathway. Angptl2$^{-/-}$ reduced oxidative stress in PQ-treated mice, as evidenced by the enhanced superoxide dismutase (SOD) activity and reduced malondialdehyde (MDA) levels in serum or lung tissue samples, which was accompanied with increased expressions of nuclear respiratory factor 2 (Nrf-2), heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO-1). PQ-induced fibrosis was also improved in Angptl2$^{-/-}$ mice by decreasing pulmonary transforming growth factor (TGF)-β1 expressions. In vitro, we found that Angptl2 knockdown-suppressed inflammation, oxidative stress and fibrosis was restored by increasing NF-κB activation in PQ-incubated A549 cells; however, the results above were significantly reversed by inactivating NF-κB using its inhibitor, Bay 11−7085 or LY2409881. Therefore, Angptl2 could provide therapeutic effects on PQ-induced acute lung injury through inhibiting inflammation, oxidative stress and fibrosis by regulating NF-κB pathway.

1. Introduction

Acute lung injury is a severe life-threatening disease, characterized by lung edema, hemorrhage, inflammatory cell infiltration, as well as diffused alveolar capillary injury on pathology, and is mainly presented as dyspnea, continuous hypoxemia, and tachycardia in clinics [1–3]. Acute lung injury, a leading cause of morbidity and mortality in critically sick patients, could result in persistent respiratory failure or even death [4]. Various factors could induce acute lung injury, including paraquat (PQ) and lipopolysaccharide (LPS) [5,6]. As reported, multiple inflammatory cells and cytokines were involved in the progression of acute lung injury [7].

Angiopoietin-like protein 2 (Angptl2) sustains tissue homeostasis by enhancing adaptive inflammation and subsequent tissue reconstruction [8]. However, excessive Angptl2 activation induced by prolonged stress accelerates breakdown of tissue homeostasis because of inflammation and irreversible tissue remodeling,

* Corresponding author.Department of Critical Care Medicine, The First Affiliated Hospital of Harbin Medical University, No. 23, YouZheng Street, Nangang District, Harbin, 150001, China.
E-mail address: zhaomingyanzz@qq.com (M. Zhao).
[1] These authors contributed equally to this work.

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promoting the progression of various diseases, such as atherosclerotic diseases, type 2 diabetes, and some cancers [9–11]. Suppressing excess Angptl2 signaling could represent novel and effective therapeutic strategies against various types of diseases and cancer [12]. However, the effects of Angptl2 on acute lung injury are yet unclear.

In the present study, we aimed to explore the role of Angptl2 in the development of acute lung injury induced by PQ by using the wild type mice (Angptl2$^{+/+}$) and Angptl2 knockout (Angptl2$^{-/-}$) in vivo, and the A549 cells with Angptl2 knockdown in vitro. We found that Angptl2 deficiency attenuates PQ-induced acute lung injury in mice by reducing inflammation, oxidative stress and fibrosis through inactivating NF-κB pathway.

2. Materials and methods

2.1. Animals and treatments

A total of 24 male, wild type (Angptl2$^{+/+}$), C57BL/6 mice (18–22 g, 8 weeks old, the Center of Animal Experiment, Heilongjiang, China) and 16 male, Angptl2 knockout (Angptl2$^{-/-}$) C57BL/6 mice (18–22 g, 8 weeks old, Cyagen Biosciences Inc., Guangzhou, China) were maintained in standard cages at controlled conditions of temperature (22 ± 2 °C) with relative humidity of 50 ± 10%, 12 h light/dark cycle and free access to water and food. All the protocols were conducted according to the ethical standards and approved by the First Affiliated Hospital of Harbin Medical University (Heilongjiang, China). The mice were randomly divided into 5 groups (n = 8): (1) wild type control (Angptl2$^{+/+}$/Con), (2) wild type PQ (Angptl2$^{+/+}$/PQ), (3) knockout control (Angptl2$^{-/-}$/Con), (4) knockout PQ (Angptl2$^{-/-}$/PQ) and (5) wild type LPS. PQ (Sigma-Aldrich, St. Louis, MO, USA), dissolved in sterile saline, was given to mice at a dose of 30 mg/kg by intraperitoneal (i.p.) injection, and the Con mice each received an i. p. injection of an equal volume of saline. The average survival rate of the mice in each group was monitored during the 14-day interval. 14 days post exposure to PQ, all mice were sacrificed for assays. Right lungs were cleared and weighed immediately to obtain the wet weight, and then put in an oven at 60 °C for 72 h to obtain the dry weight. The ratio of the wet lung to dry lung was evaluated. Peripheral blood was also collected to obtain serum for biochemical index analysis.

For LPS group, mice were treated once by intratracheal instillation with 5 mg/kg of LPS (Sigma Aldrich) in saline. After LPS treatment for 6 h, mice were sacrificed and lung tissues were isolated.

2.2. Cells and culture

Human lung cancer cell line A549 was purchased from KeyGen Biotech (Nanjing, China). The cells were cultured in RPMI-1640 containing 10% calf serum in an environment with 5% CO_2 at 37 °C. NF-κB GFP Reporter Plasmid (pGMNF-κB-GFP, 5′-TAGCAAAATAGGCTGTCCC-3′) was obtained from ZYbscience (Shanghai, China). Angptl2 siRNA and the negative control (NC) siRNA sequences were designed and synthesized by Shanghai Generay Biotechh Co., Ltd (Shanghai, China). Lipofectamine 2000 (Invitrogen, USA) was used for cell transfection following the manufacturer's protocols. Bay 11–7085 and LY2409881 were purchased from Selleckchem (USA).

2.3. Collection of bronchoalveolar lavage fluid (BALF) and analysis

BALF was collected as previously recorded [13]. Then, BALF was mixed well and centrifuged (1500 rpm, 4 °C) for 10 min. Supernatants were stored at −80 °C for total protein analysis and cytokine analysis. Total protein levels in BALF were assessed using Coomassie Brilliant Blue G-250 kits (Solarbio, Beijing, China) following the manufacturer's protocols. Pellets were prepared for inflammatory cell counts with a hemocytometer.

2.4. Measurements of cytokines, SOD and MDA

IL-1β, TNF-α and IL-6 levels in serum or BALF were measured using enzyme-linked (ELISA) kits (Bioss, Beijing, China) following the manufacturer's instructions. MDA levels and SOD activity in serum or lung tissues were determined using commercial kits (Beyotime, Nantong, China) following the manufacturer's instructions.

2.5. Isolation of RNA and real-time PCR and western blot analysis

Trizol (Invitrogen, United States) was used to extract and purify the total RNA from lung tissues or cells. The protein extraction was from lung tissue samples or cells using lysis buffer (KeyGen Biotech). Then, standard protocols were performed for RT-qPCR and western blot analysis [14]. The sequences for RT-qPCR and primary antibodies for western blot were listed in Supplementary table 1 and table 2.

2.6. Histopathology

Left upper lung tissues were fixed with 10% neutral formalin, embedded in paraffin, and sliced (5 μm thickness). Histology of lung was examined under the microscope with hematoxylin-eosin (H&E) staining. We also performed Masson's trichrome staining to evaluate fibrosis (collagen fibers) [15].

2.7. Immunohistochemistry (IHC)

Paraffin sections (4 μm thick) of mouse lungs were deparaffinized prior to antigen retrieval. Sections were blocked with normal goat plasma for 20 min at 37 °C and then incubated overnight at 4 °C with primary antibodies against the following: Angptl2 antigen (dilution 1:200; Abcam) and TGF-β1 antigen (dilution 1:100; Abcam), which was followed by incubation with secondary antibody (KeyGen Biotech) for 30 min. Diaminobenzidine (DAB, KeyGen Biotech) and hematoxylin were used for color development and counterstaining. The slides were examined under a light microscope.

2.8. Immunofluorescence (IF)

Protein expression in cells was evaluated by IF staining using TGF-β1 antigen (dilution 1:100; Abcam) diluted with 2% bovine serum albumin (Sigma) in PBS as described earlier [16]. The fluorescence signal was observed using a fluorescence microscope.

2.9. DCF-DA analysis

The levels of ROS were detected with a ROS assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions. The fluorescence signal was observed using a fluorescence microscope.

2.10. Statistical analysis

The data are presented as the means ± SEM. The differences between multiple groups were analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. $P < 0.05$ was considered statistically significant.

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3. Results

3.1. PQ-induced mice show over-expression of Angptl2 in lung tissue samples

We first calculated the expression levels of Angptl2 in PQ- or LPS-induced lung tissue samples. As shown in Fig. 1A, PQ treatment markedly increased Angptl2 expressions from mRNA and protein levels. IHC staining also showed that expression of Angptl2 was markedly increased in PQ group (Fig. 1B). In LPS-treated mice, we also found that Angptl2 was highly expressed in lung tissue samples compared with the Con group (Fig. 1C and D). In vitro, PQ-stimulated A549 cells exhibited higher levels of Angptl2 than that in the Con group (Fig. 1E). Consistently, Angptl2 expression was significantly up-regulated by LPS stimulation in A549 cells (Fig. 1F). The results above indicated that Angptl2 played an essential role in pulmonary injury induced by PQ or LPS.

3.2. Angptl2 deficiency reduces inflammation and oxidative stress in lung of PQ-induced mice

As shown in Fig. 2A, PQ treatment led to severe mortality of mice within 14 days. Compared with Angptl2$^{+/+}$/PQ group, survival rate was increased in Angptl2$^{-/-}$ mice exposure to PQ. H&E staining showed diffuse alveolar collapse and thickening in lung tissue sections of Angptl2$^{+/+}$/PQ group, which were attenuated in Angptl2$^{-/-}$/PQ group (Fig. 2B). PQ-induced increase of lung wet/dry (W/D) ratio was markedly reduced by Angptl2$^{-/-}$ (Fig. 2C). We observed a significant increase of total

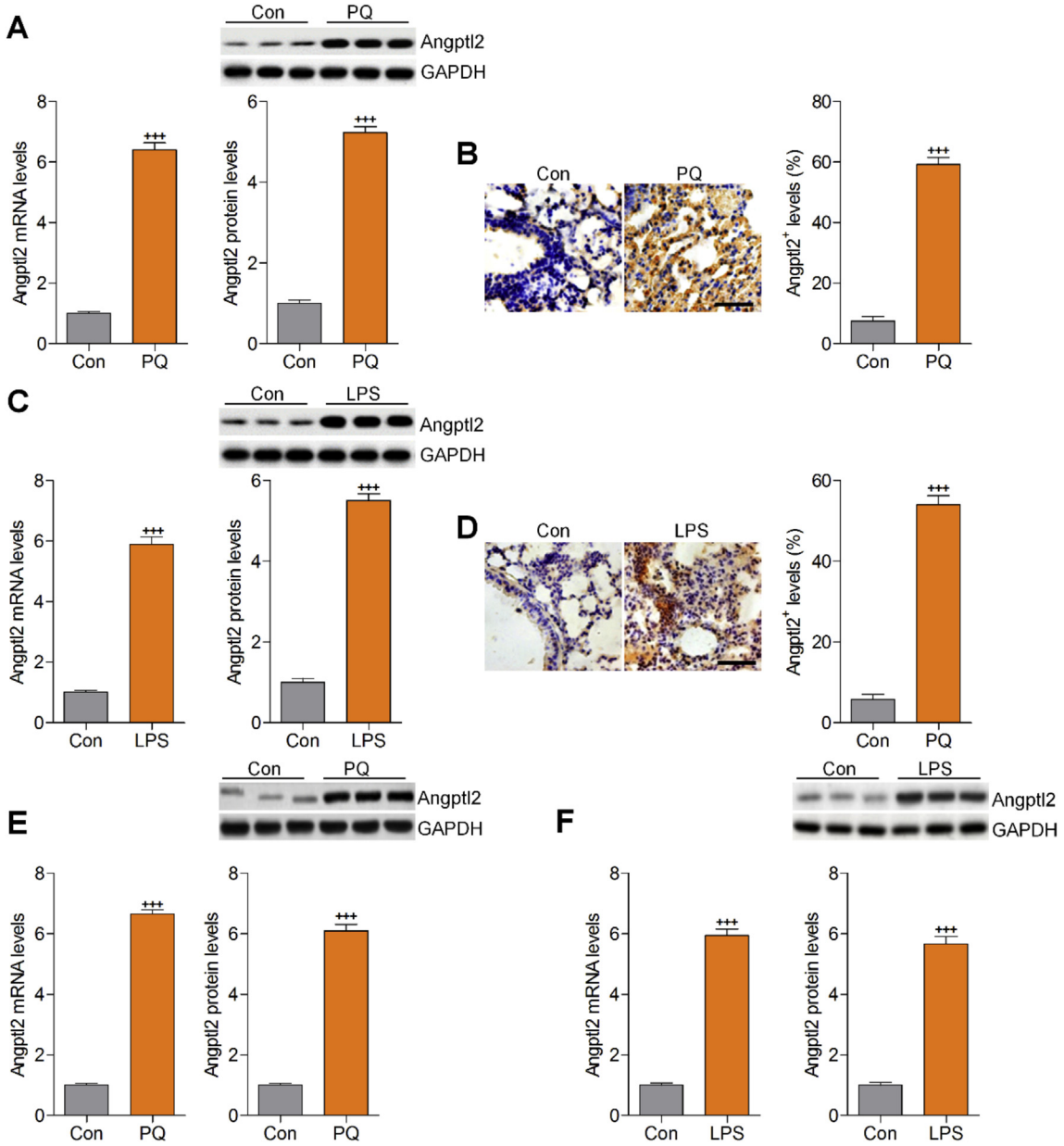

Fig. 1. PQ-induced mice shows over-expression of Angptl2 in lung tissue samples. (A) RT-qPCR and western blot analysis of Angptl2 in lung tissue samples of PQ-induced mice. (B) IHC staining of Angptl2 in pulmonary tissue sections. Scale bar = 50 μm. (C) RT-qPCR and western blot analysis of Angptl2 in lung tissue samples of LPS-treated mice. (D) IHC staining of Angptl2 in pulmonary tissue sections. Scale bar = 50 μm. (E) A549 cells were treated with PQ (300 μM) or (F) LPS (100 ng/ml) for 24 h. Then, all cells were harvested for RT-qPCR and western blot analysis of Angptl2. All data are shown as the mean ± SEM (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ versus Con group.

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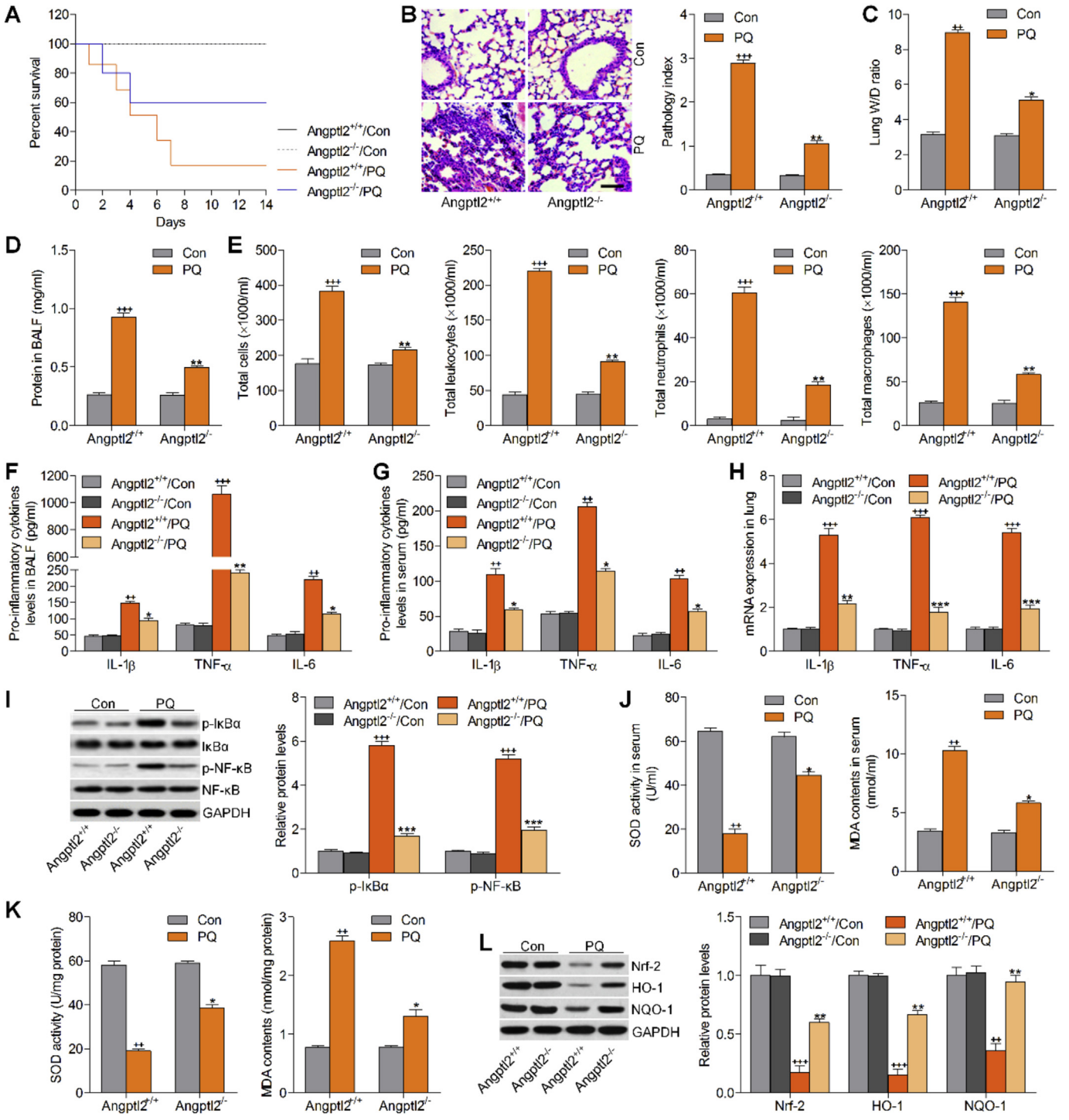

Fig. 2. Angptl2 deficiency reduces inflammation and oxidative stress in lung tissue samples of PQ-induced mice. (A) Survival rate analysis. (B) HE staining of lung tissue sections. Scale bar = 50 μm. (C) Changes of lung W/D. (D) Total protein in BALF. (E) Determination of total number and the number of inflammatory cells (leukocytes, neutrophils and macrophages) in the BALF. (F,G) The contents of IL-1β, TNF-α and IL-6 in BALF and serum of mice were assessed. (H) RT-qPCR analysis of IL-1β, TNF-α and IL-6 in pulmonary tissues. (I) Western blot analysis of p-IκBα and p-NF-κB in pulmonary tissues. SOD activity and MDA levels in (J) serum and (K) lung tissue samples were measured. (L) Western blot analysis of Nrf-2, HO-1 and NQO-1 in pulmonary tissues. All data are shown as the mean ± SEM (n = 6). $^{++}p < 0.01$ and $^{+++}p < 0.001$ versus Angptl2$^{+/+}$/Con group. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus Angptl2$^{+/+}$/PQ group.

protein levels in BALF induced by PQ, while being attenuated in Angptl2$^{-/-}$ mice (Fig. 2D). The results indicated that the numbers of total cells, total leukocytes, neutrophils and macrophages in BALF all significantly up-regulated after PQ injection, but were all reduced by Angptl2 knockout (Fig. 2E). ELISA and RT-qPCR analysis showed that the levels of pro-inflammatory cytokines, including IL-1β, TNF-α and IL-6 in BALF, serum and lung tissue samples significantly increased after exposure to PQ, but were markedly decreased in Angptl2$^{-/-}$ mice (Fig. 2F–H). Western blotting demonstrated

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that the expressions of p-IκBα and p-NF-κB were significantly enhanced after PQ exposure, which was apparently reduced by Angptl2$^{-/-}$ (Fig. 2I). As shown in Fig. 2J and K, serum and pulmonary SOD activities were evidently down-regulated by PQ treatment. In contrast, MDA levels were highly induced by PQ treatment both in serum and lung tissue of mice. However, Angptl2-knockout reversed the changes of SOD and MDA induced by PQ. PQ exposure decreased Nrf-2, HO-1 and NQO-1 expressions all dramatically. Angptl2-knockout strengthened Nrf-2, HO-1 and NQO-1 protein levels after PQ exposure (Fig. 2L).

3.3. Angptl2 knockout reduces fibrosis in lung of PQ-induced mice

Masson's trichrome staining indicated that PQ-induced fibrosis was alleviated by Angptl2$^{-/-}$ (Fig. 3A). Also, TGF-β1 expressions

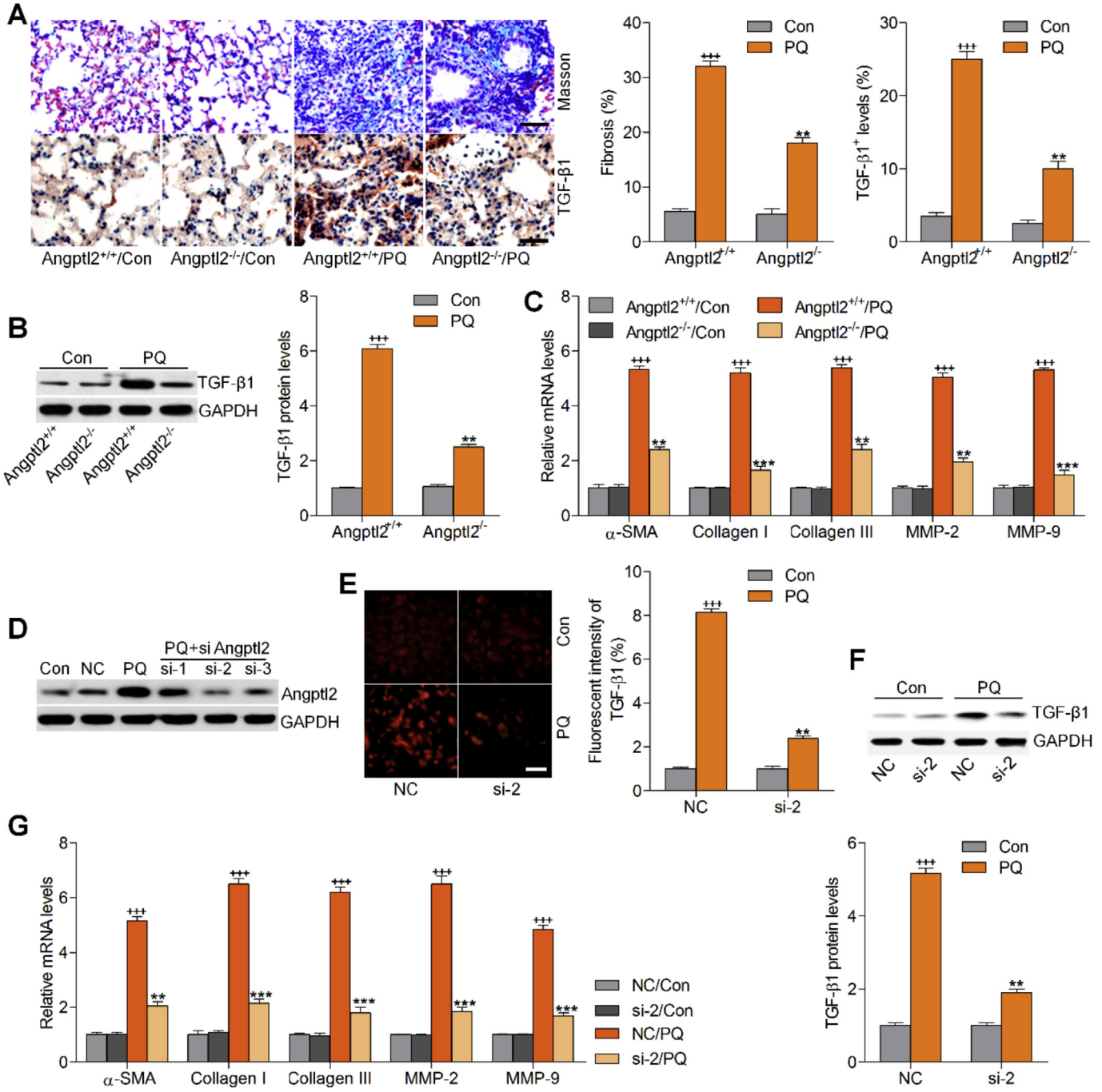

Fig. 3. Angptl2 knockout reduces fibrosis in lung of PQ-induced mice. (A) Representative images of Masson's trichrome staining and IHC analysis of TGF-β1 in lung tissue sections. Quantification of fibrosis and TGF-β1 levels was exhibited. Scale bar = 50 μm. (B) Western blot analysis of TGF-β1 in lung tissues. (C) RT-qPCR analysis of α-SMA, Collagen I, Collagen III, MMP-2 and MMP-9 in pulmonary tissue samples. $^{++}p < 0.01$ and $^{+++}p < 0.001$ versus Angptl2$^{+/+}$/Con group. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus Angptl2$^{+/+}$/PQ group. (D) A549 cells were transfected with negative control (NC) or Angptl2 siRNA sequences for 48 h, followed by 300 μM of PQ treatment for another 24 h Angptl2 protein levels were measured using western blot analysis. A549 cells were transfected with NC or Angptl2 siRNA (si-2) for 48 h, and then cells were incubated with PQ for another 24 h (E) IF and (F) western blot analysis were used to assess Angptl2 expressions. Scale bar = 50 μm. (G) RT-qPCR analysis of α-SMA, Collagen I, Collagen III, MMP-2 and MMP-9 in cells. $^{+++}p < 0.001$ versus NC/Con group. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus NC/PQ group. All data are shown as the mean ± SEM (n = 6).

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induced by PQ were down-regulated by Angptl2-deficiency (Fig. 3A and B). RT-qPCR analysis indicated that mRNA expression levels of α-SMA, Collagen I, Collagen III, MMP-2 and MMP-9 in lung tissues were markedly induced by PQ, while being reversed in Angptl2$^{-/-}$ mice (Fig. 3C). The role of Angptl2 in regulating fibrosis was further investigated in A549 cells. Angptl2 was successfully knockdown after transfection with its siRNA sequences, especially the second one (si-2) (Fig. 3D). Next, IF staining and western blot analysis showed that TGF-β1 was highly induced by PQ, whereas being reduced by Angptl2-knockdown (Fig. 3E and F). Further, PQ stimulated the mRNA levels of α-SMA, Collagen I, Collagen III, MMP-2 and MMP-9 in A549 cells, which were attenuated by Angptl2 silence (Fig. 3G).

3.4. Angptl2 knockdown alleviates PQ-induced inflammation, oxidative stress and fibrosis through inactivating NF-κB

Angptl2 has been suggested to play an essential role in regulating inflammation [8–10]. Therefore, we attempted to further reveal the underlying molecular mechanisms. Here, NF-κB phosphorylation was enhanced by transfecting pGMNF-κB-GFP in cells (Fig. 4A). Fig. 4B and C suggested that NF-κB activation was inhibited by the treatment of Bay 11–7085 and LY2409881, important inhibitors of NF-κB. Following, we found that Angptl2

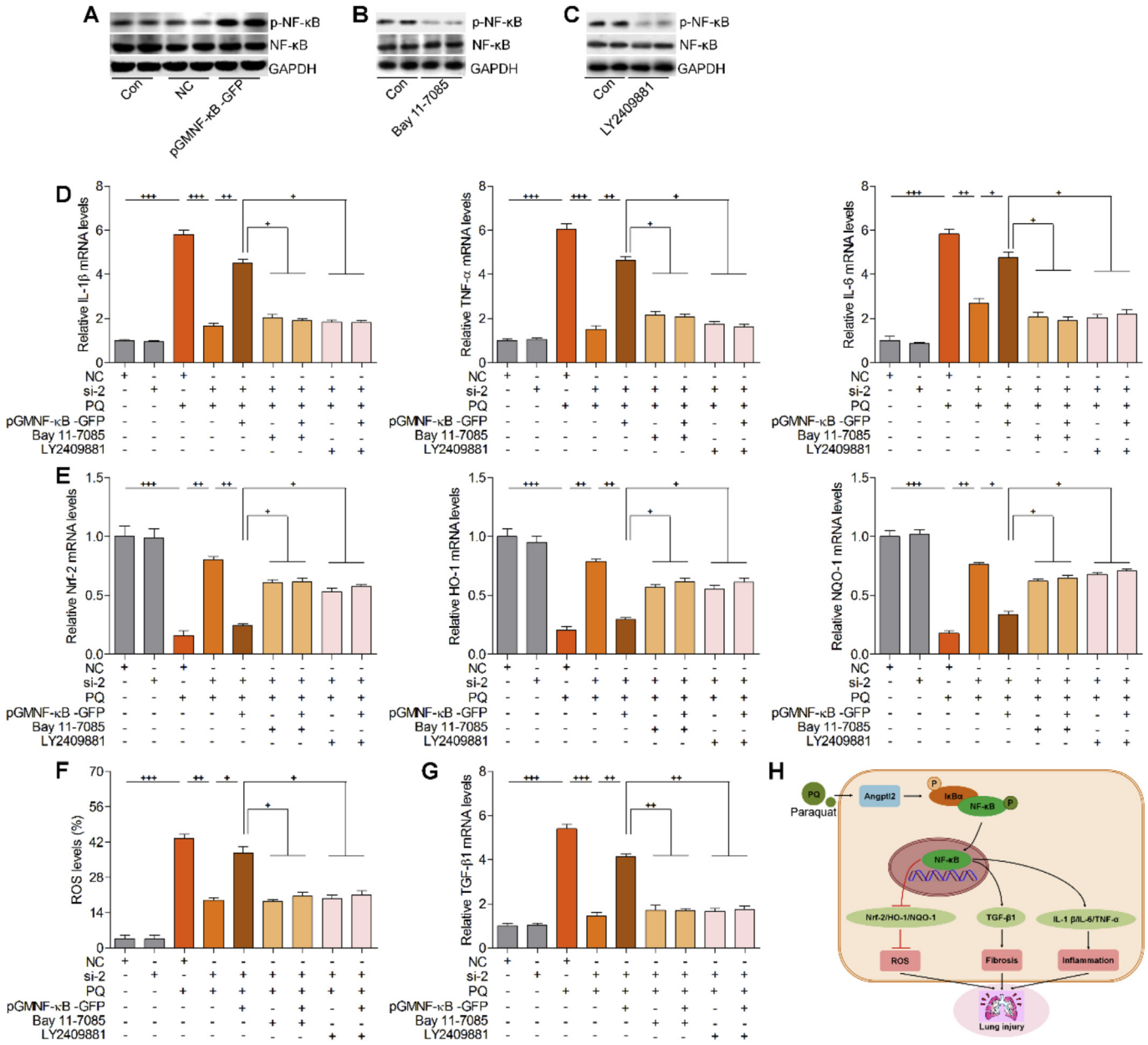

Fig. 4. Angptl2 knockdown alleviates PQ-induced inflammation, oxidative stress and fibrosis through inactivating NF-κB. (A) A549 cells were transfected with pGMNF-κB-GFP for 48 h to over-express p-NF-κB. The transfection efficiency was assessed using western blot analysis. A549 cells were treated with NF-κB inhibitors of (B) Bay 11–7085 (10 μM) and (C) LY2409881 (30 nM) for 2 h, followed by western blot analysis of p-NF-κB. A549 cells were pre-treated with Bay 11–7085 or LY2409881 for 2 h, followed by transfection with Angptl2 siRNA (si-2) and/or pGMNF-κB-GFP for 48 h. Then, all cells were incubated with PQ for an additional 24 h. RT-qPCR analysis of (D) IL-1β, TNF-α and IL-6, and (E) Nrf-2, HO-1 and NQO-1 in cells. (F) DCF-DA analysis was used to measure ROS generation in cells. (G) RT-qPCR analysis of TGF-β1 in cells. (H) A model depicting Angptl2 promoted paraquat (PQ)-induced lung injury by enhancing inflammation, oxidative stress and fibrosis through activating NF-κB pathway. All data are shown as the mean ± SEM (n = 6). $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$.

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knockdown-reduced expressions of IL-1β, TNF-α and IL-6 in PQ-treated cells were diminished by over-expressing p-NF-κB. However, pre-treatment with Bay 11–7085 and LY2409881 could reduce pGMNF-κB-GFP-induced over-expression of IL-1β, TNF-α and IL-6 in cells treated as shown in Fig. 4D. Further, over-expressing p-NF-κB decreased Nrf-2, HO-1 and NQO-1 expressions in PQ-treated A549 cells co-transfected with Angptl2 siRNA. Bay 11–7085 or LY2409881 pre-treatment significantly restored Nrf-2, HO-1 and NQO-1 mRNA levels compared to PQ-treated A549 cells, which were transfected with Angptl2 siRNA and/or pGMNF-κB-GFP (Fig. 4E). Inversely, Angptl2 knockdown-decreased ROS level was recovered in p-NF-κB-over-expressed cells after PQ stimulation, which was, apparently, restrained by Bay 11–7085 or LY2409881 pre-treatment (Fig. 4F). In Fig. 4G, we observed a significant increase of TGF-β1 induced by p-NF-κB over-expression in PQ-treated cells transfected with Angptl2 siRNA. However, Bay 11–7085 or LY2409881 pre-treatment repressed the up-regulation of TGF-β1 caused by pGMNF-κB-GFP. Therefore, the findings above indicated that Angptl2-promoted inflammation, oxidative stress and fibrosis induced by PQ was associated with the activation of NF-κB.

4. Discussion

Lung tissues are the main target organ of PQ poisoning. And PQ-induced lung injury is characterized by pulmonary edema, fibrosis, and respiratory failure [17,18]. The progression of the injury is complex, and its detailed molecular mechanisms have not been fully understood. Inflammation is reported to be a major reason of PQ-induced acute lung injury [19]. In the present study, we found that PQ-treated mice showed a significant high expression of Angptl2 in lung tissue samples. Angptl2 plays critical roles in various diseases through regulating inflammatory response [8–11,20]. Angptl2-deficiency attenuated PQ-induced lung injury, evidenced by the improved histological alterations, which might be associated with the reduction of inflammation, oxidative stress and fibrosis regulated through inactivating NF-κB.

Infiltration of macrophages and neutrophils from the alveoli to BAL is known to be essential for inducing lung inflammation by various factors, such as PQ exposure [21,22]. Here, we found that Angptl2-knockout decreased the protein leakage in BALF and markedly attenuated the lung infiltration of inflammatory cells in PQ-induced acute lung injury in mice. In addition, the expressions of pro-inflammatory cytokines, including IL-1β, TNF-α and IL-6, are important for activating inflammatory cells after acute lung injury [23]. Accordingly, IL-1β, TNF-α and IL-6 were over-released in blood and lung tissue of PQ-poisoned animals [24]. IL-1β, TNF-α and IL-6, produced by diverse cells, such as monocytes, fibroblasts and macrophages, are involved in various inflammatory lung diseases [25]. Suppressing the expression of these pro-inflammatory cytokines has become an essential therapeutic target to prevent lung diseases [26]. The p-p65 subunit of NF-κB could translocate into the nucleus, resulting in the release of a large amount of inflammatory regulators, enhancing inflammation in the lung and induces acute lung injury [27]. In our present study, we confirmed that PQ induced over-expression of IL-1β, TNF-α and IL-6, and the activation of IκBα/NF-κB pathway, which were in line with previous studies [24]. However, Angptl2-knockout alleviated the inflammatory response induced by PQ. As reported before, Angptl2-deficiency attenuated inflammation, alleviating atherosclerosis progression [28]. Therefore, suppressing Angptl2 could alleviate PQ-induced lung injury by reducing inflammation through the inactivation of IκBα/NF-κB.

Oxidative stress is another mechanism associated with PQ-induced lung injury. Free radicals are overproduced, while anti-oxidants are depleted in PQ-treated animals [29]. PQ stimulation induces the accumulation of free radicals, resulting in lipid peroxidation and the over-production of oxidation markers such as MDA. Inversely, the activities of anti-oxidant enzymes, such as SOD, are suppressed, and the balance is disturbed [30]. Nrf-2 signaling pathway is an important pathway that counteracts oxidative stress [31]. Angptl2 induced oxidative stress, creating a microenvironment, which enhances methylation of gene coding for DNA repair enzymes [32]. In our study, we found that PQ-induced oxidative stress was attenuated by Angptl2-ablation in mice, as supported by the reduced MDA levels and enhanced SOD activities in serum and lung tissue samples, as well as the up-regulation of Nrf-2, HO-1 and NQO-1. HO-1 and NQO1, oxidative-stress responsive enzymes, are significant down-streaming targets of Nrf-2 [33]. Our results above provided the evidence that Angptl2 deficiency-alleviated lung injury was associated with the repression of oxidative stress.

PQ exposure could induce myofibroblast infiltration, collagen accumulation and the differentiation of fibroblasts in alveolar septum [34]. The process might be elevated by various pro-fibrotic regulators, including TGF-β1, collagens, α-SMA and MMPs [35]. Angptl2 increases kidney fibrosis via accelerating TGF-β signaling in renal disease [36]. In addition, Angptl2 silence decreases TGF-β1-induced fibrogenesis in cardiac fibroblasts [37]. In the study, we found that Angptl2-knockout attenuated PQ-induced fibrosis formation, as evidenced by the reduced mRNA expressions of α-SMA, Collagen I, Collagen III, MMP-2 and MMP-9. Consistent with previous studies, the process regulated by Angptl2 might be associated with the reduction of TGF-β1 [36,37].

Oxidative stress could facilitate inflammatory response, which in turn exacerbates oxidative stress, contributing to excessive ROS generation in various types of cells after stimuli [38]. Inflammatory response could promote fibrosis in liver disease. And mast cells promote fibrosis by recruiting inflammatory cells [39]. NF-κB could modulate ANG II-induced renal fibrosis by regulating SMAD7, a down-streaming signal of TGF-β1 [40]. In addition, NF-κB plays an important role in indoxyl sulfate-induced fibrotic gene expression [41]. Here in our study, we found that Angptl2-regulated lung injury induced by PQ was dependent on the activation of NF-κB. Promoting NF-κB activity could diminish the role of Angptl2-knockdown in attenuating inflammation, oxidative stress and TGF-β1 expressions in PQ-treated cells, which, however, were reversed by blocking NF-κB activation by using its inhibitors. Due to a possible circle relationship between inflammation and oxidative stress/fibrosis, further study is still required in future to comprehensively reveal the underlying mechanism by which Angptl2 regulates pulmonary injury development.

In summary, our study indicated that mice with acute lung injury showed a significant increase in Angptl2 expression. As expected, suppressing Angptl2 could alleviate lung injury induced by PQ through suppressing inflammation, oxidative stress and fibrosis, which was notably regulated by NF-κB activity (Fig. 4H). This study demonstrated that targeting Angptl2 represents an effective therapeutic strategy for treatment of acute lung injury.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.05.186.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.05.186.

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