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Candida albicans-induced acute lung injury through activating several inflammatory signaling pathways in mice

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ABSTRACT

Candida albicans infection-induced acute lung injury is one of the most prevalent diseases in immunosuppressive individual. Nevertheless, the mechanism by which *Candida albicans* induced acute lung injury remains unclear. The present study investigated the mechanism by which *Candida albicans* induced acute lung injury in mice. Mice were randomly divided into four groups and intratracheally injected with 60 μl *Candida albicans* (10^6 CFU) or normal saline. Half of the mice were sacrificed at 6 h after *Candida albicans*. The rest of the mice for survival test were observed until 7 d after *Candida albicans*. As expected, immunosuppression aggravated *Candida albicans*-induced acute lung injury and death in mice. Additionally, *Candida albicans* infection elevated mRNA levels of pro-inflammatory and chemokines in lungs and the levels of IL-6, IL-1β and IL-17 in serum. Further study showed that *Candida albicans* promoted nuclear translocation of NF-κB p50 and p65 subunits in pulmonary epithelial cells and interstitial cells. *Candida albicans* induced pulmonary p38, ERK1/2 and Akt phosphorylation in normal and immunosuppressive mice. Moreover, *Candida albicans* infection activated pulmonary STAT3 signaling in normal and immunosuppressive mice. Overall, these results suggest that *Candida albicans* induced acute lung injury and death may be through activating several inflammatory signaling pathways.

1. Introduction

The fungus *Candida albicans* is a common member of the human gut microbiota. *Candida albicans* is a commensal organism that can be frequently encountered in the gastrointestinal tract, urogenital tract, mouth, skin and respiratory of healthy adults [1,2]. *Candida albicans* is also an opportunistic fungal pathogen of humans and other mammals. It can switch from benign commensal organism to pathogenic pathogen in hosts under a variety of conditions, such as HIV/AIDS infection, excessive use of broad spectrum antibiotics, congenital immunodeficiency disease, chemotherapy, radiotherapy and immunosuppressive therapies [3–5]. Fungal infections, especially *Candida albicans* infection, become highly prevalent because of the growing populations of immunocompromised individuals in the past two decades [6]. *Candida albicans* infection was approximately 30% of patients with nosocomial bloodstream infection in immunocompromised individuals, and its reported association with such infections appears to be increasing [7–9]. Moreover, *Candida albicans* infection was also a main cause of morbidity and mortality in immunocompromised individuals [10]. Pulmonary candidiasis is a type of lung infection usually caused by *Candida albicans*. Pulmonary candidiasis was one of the most prevalent diseases in patients with candidiasis [11–13]. About one-third of the patients with pulmonary candidiasis required admission to the intensive care unit (ICU) or mechanical ventilation, and the overall in-hospital mortality were significantly higher as compared with patients with non-*Candida albicans* fungal infections [14,15]. Thus, the mechanism by which pulmonary candidiasis caused by *Candida albicans* induced acute lung injury in immunosuppressive individual remains to be explored in animal experiments.

A number of studies found that *Candida albicans* infection increased the production of pro-inflammatory cytokines and chemokines, such as interleukin (IL)-6 and keratinocyte chemoattractant (KC), by host leukocytes [16–19]. A case-control study demonstrated that the levels of inflammatory cytokines were increased in patients with candidiasis as compared with in controls [20]. Indeed, numerous animal studies showed that pro-inflammatory cytokines and chemokines played an

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important role in acute lung injury [21,22]. The inhibition of several inflammatory signaling pathways significantly protected against inflammation-induced acute lung injury in rodent animals [23,24]. Nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase/protein Kinase B (PI3K/Akt) pathways were toll-like receptor 4 (TLR4) downstream inflammatory signaling pathways. Nevertheless, it remains need to explore whether these inflammatory signaling pathways involve in *Candida albicans* infection induced acute lung injury in immunosuppressive mice.

The objective of the present study is to investigate the mechanism of which *Candida albicans* infection induced acute lung injury in immunosuppressive mice. The present study found that immunosuppression treatment aggravated *Candida albicans*-induced acute lung injury and death in mice. *Candida albicans* induced the release of pro-inflammatory cytokines and chemokines. Additionally, pulmonary NF-κB, MAPK p38, and PI3K/Akt signaling pathways were activated in *Candida albicans*-treated mice. Taken together, the present study suggests that *Candida albicans* induced acute lung injury and death at least partially through activating pulmonary several inflammatory signaling pathways.

2. Material and methods

2.1. Chemicals and reagents

Candida albicans was purchased from Sigma Chemical Co. (St. Louis, MO). Nuclear factor-kappa B (NF-κB) p65/p50, phosphor-p38 (pp38), phosphor-extracellular regulated protein kinases 1/2 (pERK1/2), ERK1/2, phosphor-c-Jun NH2-terminal kinase (p-JNK), JNK, phosphor-AKT (p-Akt), Akt, β-actin and Lamin A/C antibodies were from Cell Signaling Technology (Beverley, MA). Chemiluminescence (ECL) detection kit was from Pierce Biotechnology (Rockford, IL). TRI reagent was from Molecular Research Center, Inc. (Cincinnati, Ohio). RNase-free DNase was from Promega Corporation (Madison, WI).

2.2. Animals and treatments

Male BALBc mice (8–10 week-old) were purchased from Beijing Vital River. The animals were housed on a standardized light-dark cycle in a controlled humidity (50 ± 5%) and temperature (23 ± 2 °C) environment. The animals took food and water freely at all times. After one week for adaptation, forty mice were randomly divided into four groups (ten mice each group). In immunosuppression (Dexa + Cy) group, all mice were intraperitoneally (i.p.) injected with dexamethasone (Dexa, 10 mg/kg) once daily for ten consecutive days and cyclophosphamide (Cy, 150 mg/kg) once daily for six consecutive days. In CA group, all mice were intraperitoneally (i.p.) injected with normal saline (NS) once daily for ten consecutive days. At the eleventh day, all mice were intratracheally injected with 60 μl *Candida albicans* (10^6 CFU). In Dexa + Cy + CA group, all mice were intraperitoneally (i.p.) injected with dexamethasone (Dexa, 10 mg/kg) once daily for ten consecutive days and cyclophosphamide (Cy, 150 mg/kg) once daily for six consecutive days. At the eleventh day, all mice were intratracheally injected with 60 μl *Candida albicans* (10^6 CFU). In control group, all mice were intraperitoneally (i.p.) injected with NS once daily for ten consecutive days. At the eleventh day, mice were intratracheally injected with a single dose of NS. The selection of the dosage and treatment time of *Candida albicans* was based on previous study [25]. The selection of the dosages and treatment time of Dexa and Cy was based on previous study [26]. All mice were sacrificed at 6 h after *Candida albicans* infection. Sera were collected for measurement of IL-6, IL-1β and IL-17. Lungs were collected for histopathology, immunohistochemistry, western blotting and real-time RT-PCR. For survival test, forty mice were randomly divided into Dexa + Cy group, CA group, Dexa + Cy + CA group and control group (ten mice each group).

Table 1
Primers for real-time RT-PCR.

Genes	Forward (5′ -3′)	Reverse (5′ -3′)
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Il-6	TTCCTCTGGTCTTCTGGAGT	TGTGACTCCAGCTTATCTCTTGG
Tgf-β	CGGGAAGCAGTGCCCGAACC	GGGGGTCAGCAGCCGGTTAC
Kc	ACTCAAGAATGGTCGCGAGG	GTGCCATCAGAGCAGTCTGT
Mcp-1	GGCTGGAGAGCTACAAGAGG	GGTCAGCACAGACCTCTCTC

Table 2
Body weight and lung weight.

	Weight (g)	Absolute lung weight (g)	Relative lung weight (%)
Control	25.9 ± 0.77	0.120 ± 0.0047	0.464 ± 0.0123
Dexa + Cy	25.5 ± 0.35	0.123 ± 0.0047	0.483 ± 0.0139
CA	26.5 ± 0.59	0.148 ± 0.0078*	0.558 ± 0.0270**
Dexa + Cy + CA	26.4 ± 0.90†	0.188 ± 0.0043††	0.716 ± 0.0398††

* $P < 0.05$.
** $P < 0.01$ as compared with Control group.
† $P < 0.05$.
†† $P < 0.01$ as compared with CA group.

Animal death was observed until 7 d after *Candida albicans* infection. All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Isolation of total RNA and real-time RT-PCR

Pulmonary RNA was extracted using TRI reagent. RNA was treated with RNase-free DNase and then was reverse-transcribed with AMV (Promega). Real-time RT-PCR was performed to measure mRNAs of inflammatory cytokines and chemokines in the lungs. Specific primers of genes were listed in Table 1. The amplification reactions were carried out on a LightCycler 480 Instrument referred to other study [27].

2.4. Western blotting

For total protein extraction, pulmonary tissue homogenate was prepared using a lysis buffer containing PMSF. After 20 min on ice, tissue homogenate was centrifuged for 15 min at 15000 rpm. Supernatant was collected and then protein concentration was measured with Lowry referred to the manufacturer's instructions. The protein sample was denatured for 10 min at 98 °C. Pulmonary nuclear protein was extracted through homogenizing 400 mg lung tissue in 5 ml lysis buffer A (0.6% NP-40, 150 mM NaCl, 10 mM Hepes-KOH pH 7.9, 1 mM EDTA, 0.5 mM PMSF). Tissue homogenate was centrifuged for 30 s at 600 rpm. Supernatant was collected and then was centrifuged for 20 min at 6000 rpm. Precipitate was collected and then was lysed with 100 μl lysis buffer B (20 mM Hepes-KOH pH 7.9, 420 mM NaCl, 1.2 mM MgCl2, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM dithiothreitol, Protease Inhbitor Cocktail and 25% glycerol). After 1 h on ice, lysis was centrifuged for 15 min at 15000 rpm. Supernatant was collected and then protein concentration was measured with Lowry referred to the manufacturer's instructions. For western blotting, same amount of protein (15–30 μg) was electrophoresed by 12.5% SDS-PAGE and transferred to a polyvinylidene fluoride membrane. For total protein, the membranes were incubated for 1–2 h with the following antibodies: p-ERK1/2, ERK1/2, p-JNK, JNK, p-p38, p38, p-Akt, Akt p-STAT3, STAT3 and β-actin. For nuclear protein, the membranes were incubated for 2 h with NF-κB p65, NF-κB p50, ROR-γt, ROR-α and Lamin A/C antibodies. The membranes were washed for three times and then were incubated with second antibody for 1–2 h. After washes in DPBS, signal was measured using an ECL luminescence.

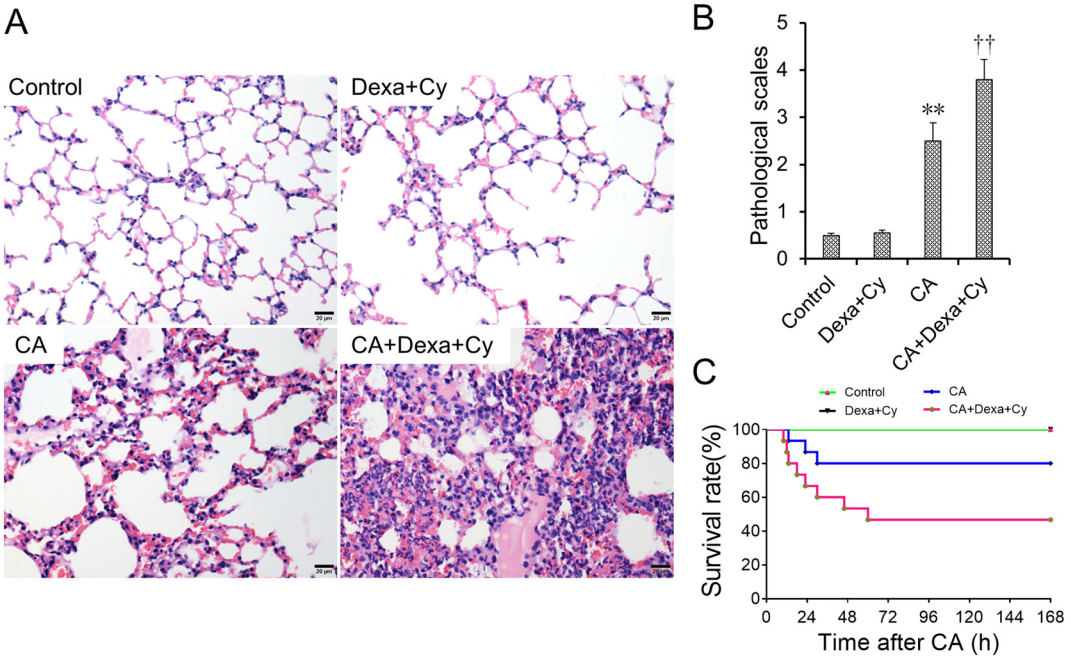

Fig. 1. *Candida albicans* infection-induced acute lung injury in normal and immunosuppressive mice. (A and B) Mice were treated as in Material and methods. All mice were sacrificed 6 h after *Candida albicans* infection. Acute lung injury was evaluated by H&E. (A) Representative photomicrographs of pulmonary histology were shown. Original magnification: × 400. (B) Pathological scores for pulmonary injury were evaluated in each slide at a magnification of 400×. Data were expressed as means ± SEM of 10 samples from 10 different mice. (C) For survival test, mice were treated as in Material and methods. Animal death was observed until 7 days after *Candida albicans* infection. $^{**}P < 0.01$ as compared with control. $\dagger\dagger P < 0.01$ as compared with CA.

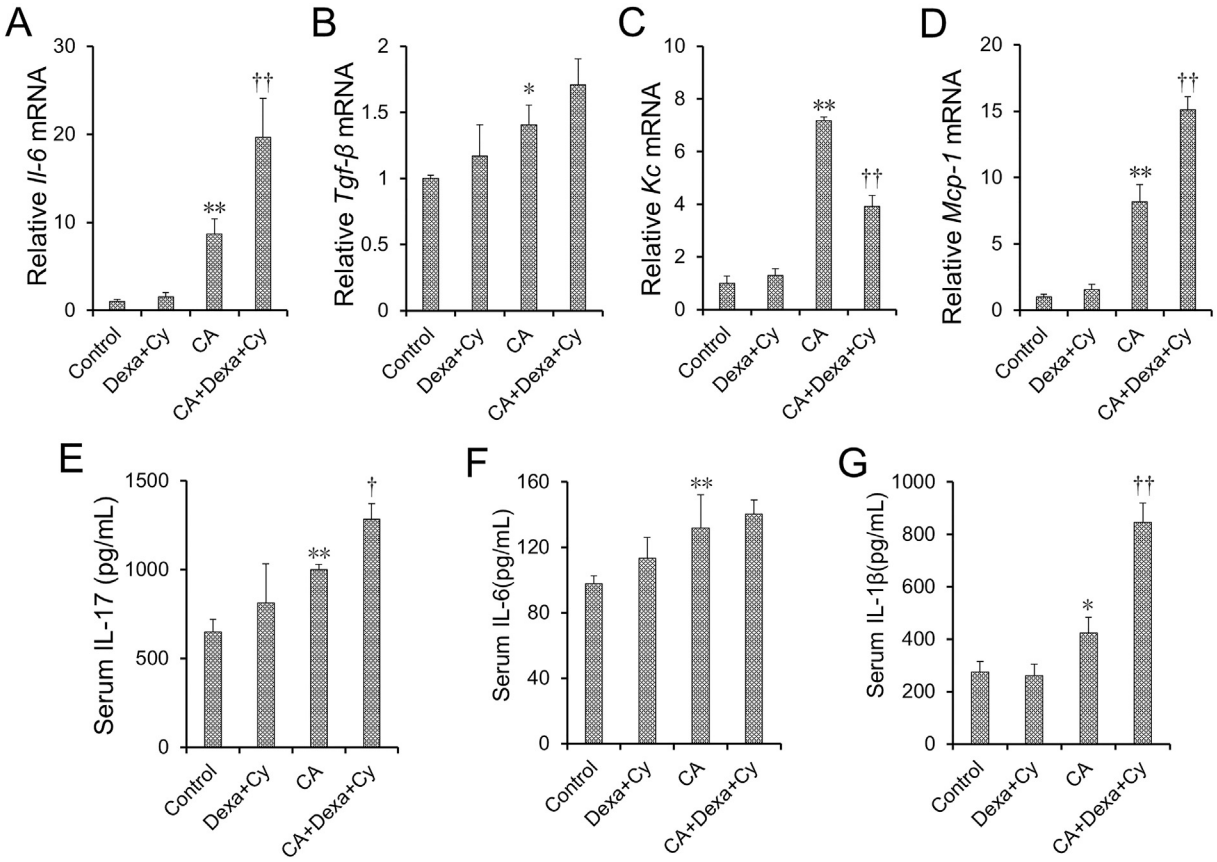

Fig. 2. *Candida albicans* infection up-regulated the expressions of pulmonary inflammatory cytokines and chemokines in normal and immunosuppressive mice. Mice were treated as in Material and methods. All mice were sacrificed 6 h after *Candida albicans* infection. (A–D) Pulmonary mRNA levels were measured by real-time RT-PCR. (A) *Il-6*. (B) *Tgf-β*. (C) *Kc*. (D) *Mcp-1*. (E-G) Serum inflammatory cytokines were measured by ELISA. (E) IL-17. (F) IL-6. (G) IL-1β. All data were expressed as means ± SEM of 10 samples from 10 different mice. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with control. $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ as compared with CA.

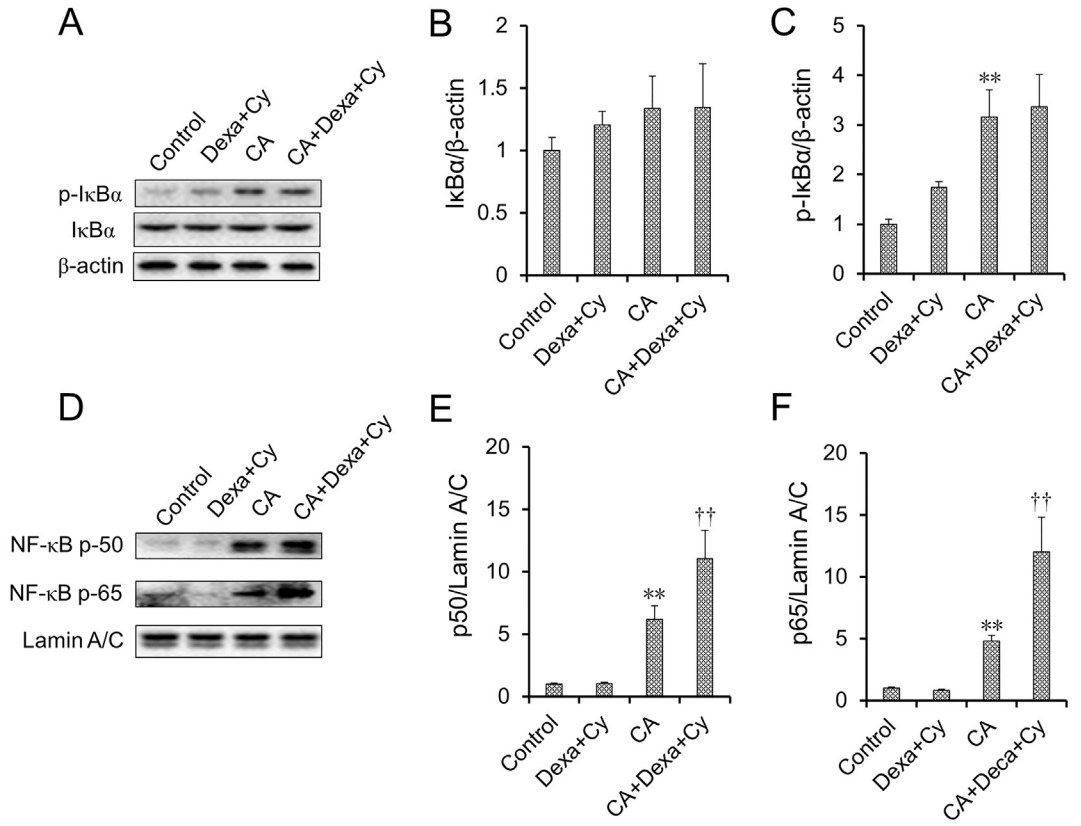

Fig. 3. *Candida albicans* infection activated pulmonary NF-κB signaling in normal and immunosuppressive mice. Mice were treated as in Material and methods. All mice were sacrificed 6 h after *Candida albicans* infection. Total and nuclear protein was measured using western blotting. (A) Representative gels for p-IκBα, IκBα and β-actin were shown. (B) Quantitative analysis of scanning densitometry for p-IκBα was performed. (C) Quantitative analysis of scanning densitometry for IκBα was performed. (D) Representative gels for NF-κB p50, p65 and Lamin A/C were shown. (E) Quantitative analysis of scanning densitometry for p50 was performed. (F) Quantitative analysis of scanning densitometry for p65 was performed.

2.5. Immunohistochemistry (IHC)

Pulmonary sections were made based on the standard protocol [27]. Section was incubated with anti-NF-κB p65/p50 and p-STAT3 monoclonal antibodies (1:200) at 4 °C overnight. The color reaction was developed with HRP-linked polymer detection system and counterstaining with hematoxylin before dehydrating in graded alcohols. p65-, p50- and p-STAT3-positive cells were counted in twelve randomly selected fields from each slide at a magnification of × 400.

2.6. Enzyme-linked immunosorbent assay

Commercial enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Abingdon, Oxon, UK) kits were used to measure serum TNF-α and KC levels referred to the manufacturer's protocol.

2.7. Statistical analysis

Quantified data were expressed as means ± S.E.M. Differences among different groups were evaluated using ANOVA. Data that were not normally distributed were assessed for significance using non-parametric tests techniques. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Candida albicans infection-induced acute lung injury in normal and immunosuppressive mice

As shown in Table 2, pulmonary weight was slightly elevated at 6 h after *Candida albicans* infection. In addition, lung coefficient also slightly increased at 6 h after *Candida albicans* infection. As expected, an infiltration of numerous inflammatory cells and thickening of the alveolar wall were observed in the lungs in *Candida albicans*-treated mice (Fig. 1A and B). Immunosuppression treatment significantly aggravated *Candida albicans*-induced pulmonary edema (data not shown). Additionally, Immunosuppression treatment significantly aggravated *Candida albicans*-induced infiltration of inflammatory cells and thickening of the alveolar wall in mice lungs (Fig. 1A and B). Survival test showed that 30% (3/10) of normal mice were dead until 72 h after *Candida albicans* infection (Fig. 1C). Of interest, 60% (6/10) of immunosuppressive mice were dead until 72 h after *Candida albicans* infection (Fig. 1C).

3.2. Candida albicans infection up-regulated the expressions of pulmonary inflammatory cytokines and chemokines in normal and immunosuppressive mice

The effects of *Candida albicans* infection on the expression of pro-inflammatory and chemokine genes in lung are analyzed. As shown in Fig. 2A–D, mRNA levels of pulmonary *Il-6* and *Tgf-β*, two pro-inflammatory genes, were significantly increased after *Candida albicans* infection in normal and immunosuppressive mice (Fig. 2A, B). Moreover, mRNA levels of pulmonary keratinocyte chemoattractant (*Kc*) and *Mcp-1*, two chemokine genes, were markedly up-regulated after *Candida albicans* infection in normal and immunosuppressive mice (Fig. 2C, D). Interestingly, mRNA levels of pulmonary *Il-6* and *Mcp-1* were further increased in immunosuppressive mice lungs as compared with normal mice lungs (Fig. 2A, D). The levels of serum IL-17, IL-6 and IL-1βwere further measured. As shown in Fig. 2E–G, The levels of serum

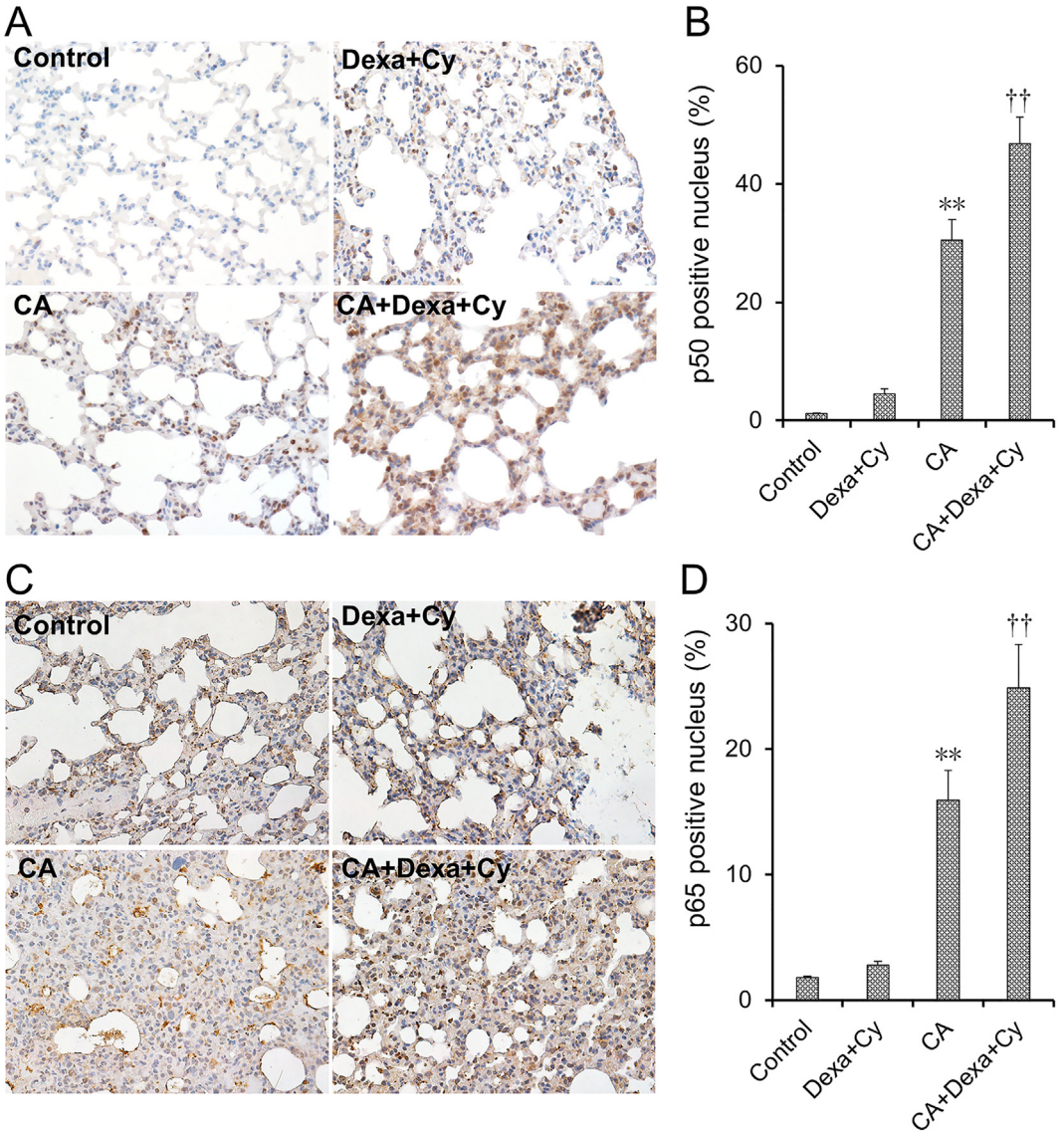

Fig. 4. *Candida albicans* infection upregulated pulmonary NF-κB p65 and p50 positive nucleus in normal and immunosuppressive mice. Mice were treated as in Material and methods. All mice were sacrificed 6 h after *Candida albicans* infection. Total and nuclear protein was measured by immunohistochemistry. (A) Representative photomicrographs of pulmonary p50 were shown. Original magnification: 400×. Arrowheads indicate p50-positive nucleus. (B) NF-κB p50 positive cells were compared among different groups. (C) Representative photomicrographs of pulmonary p65 were shown. Original magnification: 400×. Arrowheads indicate p50-positive nucleus. (D) NF-κB p65 positive cells were compared among different groups. All experiments were duplicated for three times. All data were expressed as means ± SEM. $^{**}P < 0.01$ as compared with control. $\dagger\dagger P < 0.01$ as compared with CA.

IL-17, IL-6 and IL-1β were significantly increased after *Candida albicans* infection in normal mice. Of interest, the levels of serum IL-17 and IL-1β were further increased after *Candida albicans* infection in immunosuppressive mice as compared with normal mice (Fig. 2E, G).

3.3. *Candida albicans* infection activated pulmonary NF-κB signaling in normal and immunosuppressive mice

The effects of *Candida albicans* on pulmonary NF-κB signaling were measured. No difference was observed on the level of pulmonary IκBα, an inhibitor protein of NF-κB, among four groups (Fig. 3A, B). However, the level of pulmonary p-IκBα was significantly increased after *Candida albicans* in normal and immunosuppressive mice (Fig. 3A, C). Additionally, the levels of pulmonary NF-κB p50 and p65 were significantly increased after *Candida albicans* in normal mice (Fig. 3D–F). Interestingly, the levels of pulmonary NF-κB p50 and p65 were further increased in immunosuppressive mice as compared with normal mice (Fig. 3D–F). Nuclear translocation of pulmonary NF-κB p50 and p65 were further analyzed using immunohistochemistry. Results showed that nuclear translocation of NF-κB p50 and p65 was mainly observed in the pulmonary epithelial cells and interstitial cells of normal and immunosuppressive mice (Fig. 4A–B). Interestingly, the number of NF-κB p50 and p65 positive nucleus was increased in immunosuppressive mice as compared with normal mice after *Candida albicans* (Fig. 4A–D).

3.4. *Candida albicans* infection activated pulmonary MAPKs signaling in normal and immunosuppressive mice

The levels of pulmonary p-p38, p38, p-ERK1/2, ERK1/2, p-JNK and JNK were measured in the lungs. As shown in Fig. 4A, there were no differences on the levels of p38, ERK1/2 and JNK in the lungs among four groups. *Candida albicans* had no effect on the expression of p-ERK1/2 and p-JNK protein (Fig. 5A, C, D). Of interest, *Candida albicans* infection significantly induced pulmonary p38 phosphorylation in

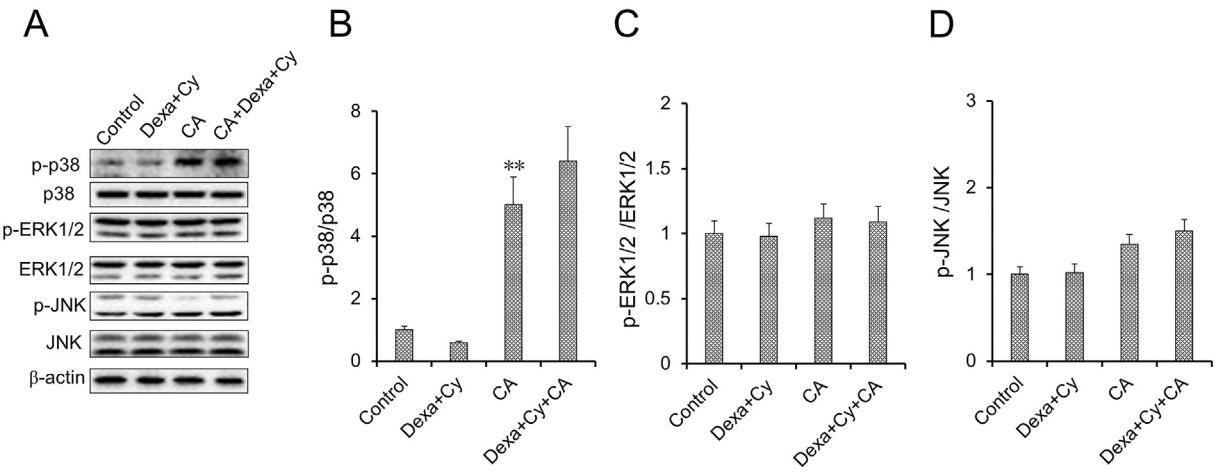

Fig. 5. *Candida albicans* infection activated pulmonary MAPKs signaling in normal and immunosuppressive mice. Mice were treated as in Material and methods. All mice were sacrificed 6 h after *Candida albicans* infection. Total protein was measured using western blotting. (A) Representative gels for p-p38, p38, p-ERK1/2, ERK1/2, p-JNK, JNK and β-actin were shown. (B) Quantitative analysis of scanning densitometry for p-p38 was performed. (C) Quantitative analysis of scanning densitometry for p-ERK1/2 was performed. (D) Quantitative analysis of scanning densitometry for p-JNK was performed. All experiments were duplicated for three times. All data were expressed as means ± SEM. $^{**}P < 0.01$ as compared with control. $\dagger\dagger P < 0.01$ as compared with CA.

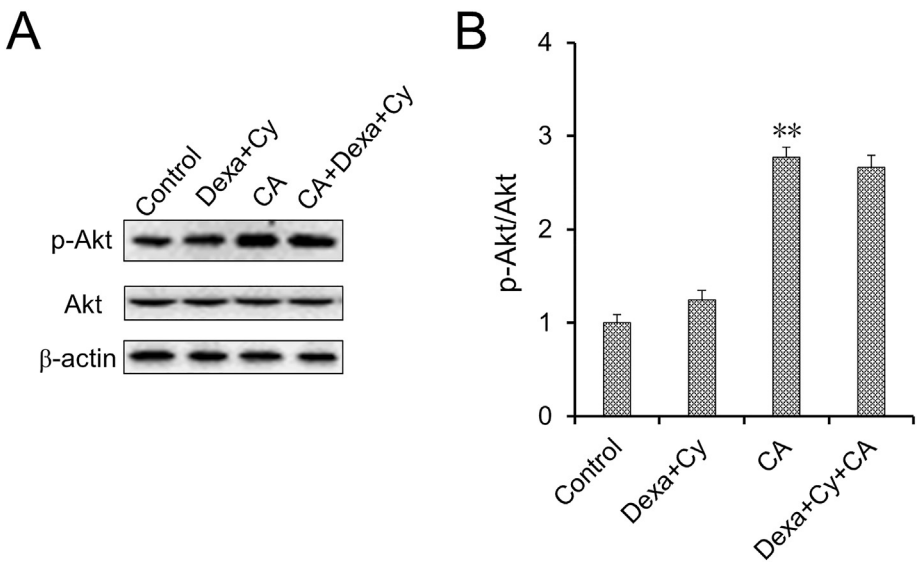

Fig. 6. *Candida albicans* infection activated pulmonary PI3K/Akt signaling in normal and immunosuppressive mice. Mice were treated as in Material and methods. All mice were sacrificed 6 h after *Candida albicans* infection. Total protein was measured using western blotting. (A) Representative gels for p-Akt, Akt and β-actin were shown. (B) Quantitative analysis of scanning densitometry was performed. All experiments were duplicated for three times. All data were expressed as means ± SEM. $^{**}P < 0.01$ as compared with control. $\dagger\dagger P < 0.01$ as compared with CA.

normal and immunosuppressive mice (Fig. 5A, B).

3.5. *Candida albicans* infection activated pulmonary PI3K/Akt signaling in normal and immunosuppressive mice

Pulmonary PI3K/Akt signaling are measured. As shown in Fig. 6, *Candida albicans* infection had no effect on the expression of Akt protein in the lungs. However, *Candida albicans* infection significantly induced pulmonary Akt phosphorylation in normal and immunosuppressive mice (Fig. 6A, B).

3.6. *Candida albicans* infection activated pulmonary STAT3 signaling in normal and immunosuppressive mice

To investigate the effect of *Candida albicans* infection on pulmonary STAT3 signaling, pulmonary pSTAT3/STAT3, ROR-γt and ROR-α were measure. As shown in Fig. 6A, B, *Candida albicans* infection had no effect on the expression of STAT3 protein in the lungs. *Candida albicans* infection significantly induced pulmonary STAT3 phosphorylation (Fig. 7A, B). Interestingly, the levels of pulmonary p-STAT3 were further increased in immunosuppressive mice as compared with normal mice (Fig. 6A, B). Moreover, the levels of nuclear ROR-γt and ROR-α in the lungs were significantly elevated at 6 h after *Candida albicans* infection in normal and immunosuppressive mice (Fig. 7A, C, D). Immunohistochemistry showed that p-STAT3 positive nucleus was mainly observed in the pulmonary epithelial cells and interstitial cells of normal and immunosuppressive mice (Fig. 8A). Interestingly, the number of p-STAT3 positive nucleus was increased in immunosuppressive mice as compared with normal mice after *Candida albicans* (Fig. 8A, B).

4. Discussion

The present study explored the mechanism by which *Candida albicans* induced acute lung injury in normal mice and immunosuppressive mice. As expected, pulmonary *Candida albicans* infection induced acute lung injury and death in mice. Immunosuppression treatment aggravated *Candida albicans*-induced acute lung injury and death in mice. Additionally, *Candida albicans* infection elevated mRNA levels of pro-inflammatory and chemokines in lungs and the levels of IL-6, IL-1β and IL-17 in serum. Further study showed that *Candida albicans* activated pulmonary NF-κB signaling, MAPKs signaling, PI3K/Akt signaling and

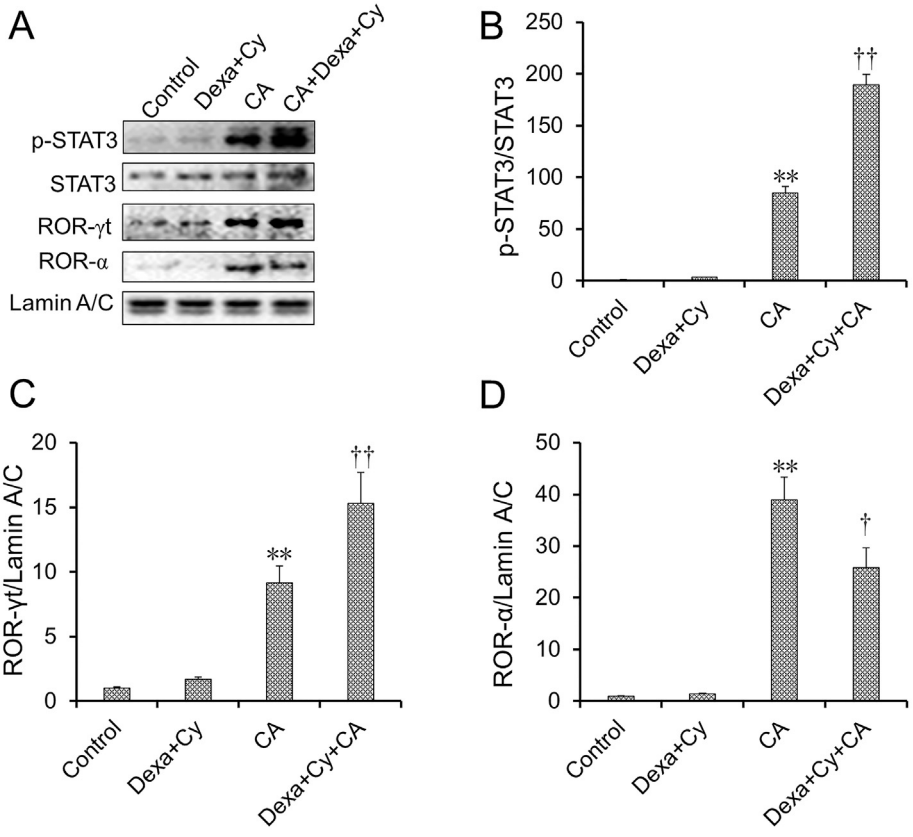

Fig. 7. *Candida albicans* infection activated pulmonary STAT3 signaling in normal and immunosuppressive mice. Mice were treated as in Material and methods. All mice were sacrificed 6 h after *Candida albicans* infection. Total and nuclear protein was measured using western blotting. (A) Representative gels for pSTAT3, STAT3, ROR-γt, ROR-α and Lamin A/C were shown. (B) Quantitative analysis of scanning densitometry for p-STAT3/STAT3 was performed. (C) Quantitative analysis of scanning densitometry for ROR-γt was performed. (D) Quantitative analysis of scanning densitometry for ROR-α was performed.

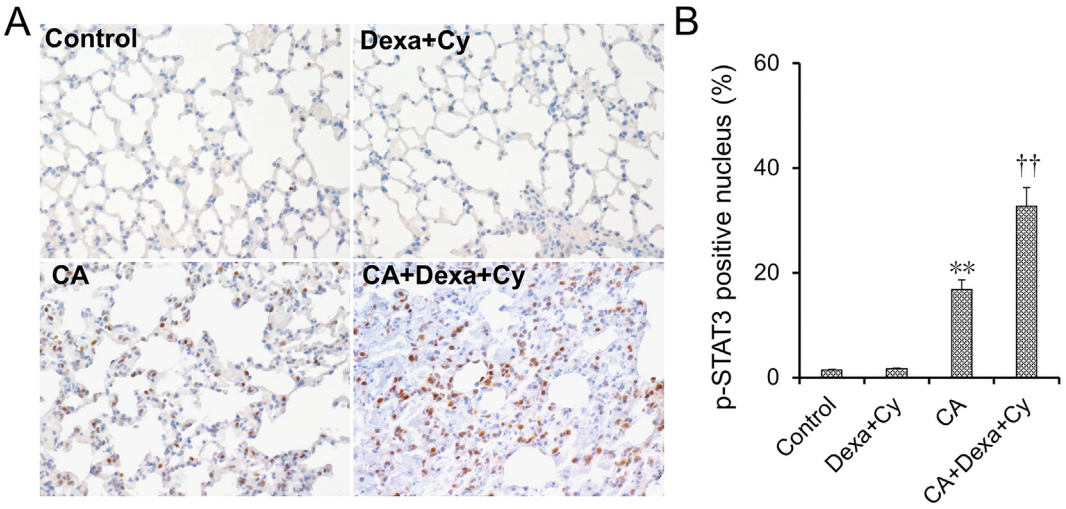

Fig. 8. *Candida albicans* infection upregulated pulmonary STAT3 positive nucleus in normal and immunosuppressive mice. (A and B) p-STAT3 was analyzed using IHC. (A) Representative photomicrographs of pulmonary p-STAT3 was shown. Original magnification: 400×. Arrowheads indicate p-STAT positive nucleus. (B) p-STAT3 positive cells were compared among different groups. All experiments were duplicated for three times. All data were expressed as means ± SEM. $^{**}P < 0.01$ as compared with control. $^{††}P < 0.01$ as compared with CA.

STAT3 signaling in normal and immunosuppressive mice. These results suggest that *Candida albicans* induced acute lung injury may be through activating several inflammatory signaling pathways.

Increasing evidences showed that systemic and pulmonary local inflammation was one of the main causes of acute lung injury. On the one hand, several studies found that pro-inflammatory cytokines IL-1β resulted in acute lung injury in rodent [28]. Anti-inflammatory cytokines IL-10 deficiency augments acute lung injury [29]. Moreover, treatment with anti-inflammatory drugs, such as obeticholic acid and vitamin D, or anti-inflammatory cytokines IL-10 alleviated xenobiotic-induced acute lung injury [23,30,31]. On the other hand, epidemiological studies demonstrated that TNF-α, IL-1β and IL-8 were significantly higher in the epithelial lining fluid and bronchoalveolar lavage in patients with acute lung injury as compared with controls [32]. Pneumonia patients with low frequency of pulmonary CD19 + CD24(hi)CD38(hi) regulatory B cells, a well-known immunocyte of secreting IL-10, have severe susceptibility to acute lung injury [33]. Indeed, human and animal studies found that serum inflammatory cytokines levels were dramatically increased in subjects with *Candida albicans* infection compared with subjects with bacterial

infection and controls [34]. The present study also found that *Candida albicans* infection elevated mRNA levels of pro-inflammatory and chemokines in lungs. Additionally, *Candida albicans* infection increased the levels of IL-6, IL-1β and IL-17 in serum. Thus, these results indicate that inflammation involved in *Candida albicans* infection induced acute lung injury.

The mechanism by which *Candida albicans* infection elevated the levels of inflammatory cytokines in serum and infected lung remains unclear. Several studies showed that *Candida albicans*-derived mannoproteins was a ligand for toll-like receptor 4 (TLR4), suggesting *Candida albicans* infection elevates the levels of inflammatory cytokines may be through activating TLR4 pathway [35,36]. NF-κB was one of downstream signaling of TLR4 pathway. Generally, NF-κB subunits are retained in the cytoplasm through binding to the inhibitor of NF-κB (IκB)-α. When IκBα is phosphorylated, NF-κB subunits, such as NF-κB p50 and p65, translocate to the nucleus to induce the expression of its downstream inflammatory genes [37,38]. The present study found that the levels of p-IκBα and nuclear NF-κB p50 and p65 in the lung were significantly increased after *Candida albicans* in normal and immunosuppressive mice. Additionally, the levels of nuclear NF-κB p50 and p65 in the lungs were higher in immunosuppressive mice compared with normal mice. These results indicate that *Candida albicans* induces the release of pro-inflammatory cytokines and chemokines partially through activating NF-κB signaling pathway. MAPKs and PI3K/Akt were other two downstream signaling pathways that regulate TLR4-mediated inflammatory genes. Phosphorylation MAPKs, including p-p38, p-ERK1/2 and p-JNK, and phosphorylation Akt translocate to the nucleus to induce the expression of its downstream inflammatory genes [39]. The present study showed that the levels of pulmonary p-p38 and p-Akt were significantly elevated in *Candida albicans* infected-mice. These results suggest that *Candida albicans* induces the release of pro-inflammatory cytokines and chemokines partially via activating MAPK p38 and PI3K/Akt signaling pathways.

Signal transducers and activators of transcription 3 (STAT3) is a pivotal transcription factor in the differentiation and maturation of Th17, an independent subset of effector T helper cells [40,41]. Phosphor-STAT3 undergoes oligomerization and up-regulates expression of retinoid-related orphan receptors (RORs), including ROR-γ and RORα, through binding to the RORs promoter site. Both phosphor-STAT3 and RORs complex to bind to IL-17 promoter region to induce Th17 cell maturation and IL-17 secretion [42,43]. Most of the studies showed that IL-17 played an important role in host defense, particularly against *Candida albicans* infection [44,45]. Mice and humans with defects in Th17/IL-17 immunity have been documented to have severe susceptibility to *Candida albicans* infection [46,47]. The present study investigated the effect of STAT3/RORs signaling on *Candida albicans* infections induced acute lung injury. Unexpectedly, the levels of nuclear phosphor-STAT3, ROR-γt and RORα were increased 6 h after *Candida albicans* infection. In addition, serum IL-17 was elevated in *Candida albicans* infected-mice. These results indicate that *Candida albicans* infection may activate host immune response. On the other hand, STAT3 is also a key transcriptional factor involved in inflammation [48,49]. Blocking of STAT3 activity attenuated ethanol-induced liver injury and LPS-induced acute lung injury by inhibition of inflammatory responses [50,51]. IL-17 is also a pro-inflammatory cytokines. Animal studies showed that IL-17 played an important role in mouse lung injury models [52,53]. Thus, these results suggest that STAT3/IL-17 pathway may partially involve in *Candida albicans* infection-induced inflammation and acute lung injury.

The present study has several flaws. Firstly, the present study had not investigated the mechanism by which *Candida albicans* induced acute lung injury through knockout mice model. Knockout mice model could help to further confirm the mechanisms and hence this investigation. Secondly, immunohistochemistry found that NF-κB p50, p65 p-STAT3 positive nucleus was mainly observed in not only the pulmonary epithelial cells but slao interstitial cells using. Thus, the present study doesn't claim what cells were injured *by Candida albicans*.

In summary, the present study found that immunosuppression treatment aggravated *Candida albicans*-induced acute lung injury and death in mice. *Candida albicans* induced the release of pro-inflammatory cytokines and chemokines in serum and infected lungs. Additionally, pulmonary NF-κB, MAPK p38, and PI3K/Akt signaling pathways were activated in *Candida albicans*-treated mice. Taken together, the present study suggests that *Candida albicans* induced acute lung injury at least partially through activating several inflammatory signaling pathways. Thus, our results indicated that anti-inflammatory treatment may be used as a potential means to treat *Candida albicans* infection-induced acute lung injury and death.

Author contributions

Participated in research design: Jia-Bin Li.

Conducted experiments: Zhi-Li Xu, Se-Ruo Li, Lin Fu, Ling Zheng, Jing Ye.

Contributed new reagents/materials/analysis tools: Jia-Bin Li.

Performed data analysis: Zhi-Li Xu.

Wrote or contributed to the writing of the manuscript: Zhi-Li Xu.

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Competing interests

The authors report no conflict of interests.

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