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Research article

Melatonin-induced upregulation of telomerase activity interferes with macrophage mitochondrial metabolism and suppresses NLRP3 inflammasome activation in the treatment of Pneumonia

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ABSTRACT

Objective: This study aims to investigate the effects of melatonin-induced upregulation of telomerase activity on mitochondrial metabolism and NLRP3 inflammasome activation in macrophages, with the ultimate goal of elucidating potential therapeutic implications for pneumonia treatment.
Materials and methods: Macrophages were treated with melatonin to assess its impact on telomerase activity. Mitochondrial function was evaluated through the measurement of reactive oxygen species (ROS) levels and cellular energy production. NLRP3 inflammasome activation was assessed by examining the production of inflammatory cytokines, such as interleukin-1β (IL-1β). The expression levels of key proteins involved in mitochondrial metabolism and NLRP3 inflammasome signaling were also analyzed.
Results: Our findings demonstrated that melatonin treatment significantly upregulated telomerase activity in macrophages. This was associated with a reduction in ROS levels and enhanced cellular energy production, indicating improved mitochondrial function. Moreover, melatonin treatment suppressed NLRP3 inflammasome activation, resulting in reduced secretion of IL-1β. The expression levels of proteins involved in mitochondrial metabolism and NLRP3 inflammasome signaling were modulated by melatonin.
Conclusion: These results suggest that melatonin-induced upregulation of telomerase activity can interfere with mitochondrial metabolism and inhibit NLRP3 inflammasome activation in macrophages. This indicates a potential therapeutic role for melatonin in the treatment of pneumonia. Understanding the molecular mechanisms underlying these effects may lead to the development of novel therapeutic strategies targeting mitochondria and NLRP3 inflammasome activation for the management of pneumonia. Further investigations are warranted to fully uncover the therapeutic potential of melatonin and its implications for pneumonia treatment.

1. Introduction

In recent years, the exploration of novel therapeutic strategies for the treatment of pneumonia has gained traction [1]. Pneumonia, an infection of the lungs, is a major global health concern and a leading cause of morbidity and mortality worldwide [2]. Bacterial pneumonia is a common pathogenic type of pulmonary inflammation, and one of the common pathogens is Klebsiella pneumoniae [3,

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4]. Klebsiella pneumoniae has a thick capsule, can parasitise the intestinal tract and upper respiratory tract mucosa, and can multiply rapidly in a variety of organs and cause disease [5]. Despite advancements in antibiotics and supportive care, the incidence and severity of pneumonia remain high, necessitating the development of innovative therapeutic approaches [6,7].

Melatonin, a hormone primarily secreted by the pineal gland, has been widely studied for its neuroprotective, antioxidant, and immunomodulatory properties [8]. Apart from its well-recognized role in regulating sleep-wake cycles, melatonin has been implicated in various physiological processes, including the immune response [9]. Recent studies have suggested that melatonin may play a crucial role in modulating host defense mechanisms against pulmonary infections, pneumonia [10,11].

Telomeres, which are repetitive DNA sequences located at the ends of chromosomes, play a vital role in maintaining chromosomal stability and integrity. Telomere shortening is associated with cellular senescence and aging, as well as the progression of various diseases, including pneumonia [12]. Telomerase, the enzyme responsible for telomere elongation, counteracts telomere shortening by adding repeated DNA sequences to the ends of chromosomes [13]. Recent studies have shown that modulation of telomerase activity can influence immune responses, including those involved in the pathogenesis of pneumonia [14]. Mitochondria, the powerhouses of the cell, are essential for cellular energy production, metabolism, and signaling. Mounting evidence suggests that mitochondrial function is closely linked to immune responses and the development of inflammatory diseases [15,16]. Dysregulation of mitochondrial metabolism can result in the accumulation of reactive oxygen species (ROS), impaired cellular energy production, and aberrant immune responses [17]. Understanding the potential role of mitochondria in pneumonia pathogenesis is crucial for identifying novel therapeutic targets [18,19].

NLRP3 (NACHT, LRR, and PYD domains-containing protein 3) inflammasome is a multi-protein complex that plays a critical role in the innate immune response and the generation of inflammatory cytokines such as interleukin-1β (IL-1β) [20]. Dysregulation of NLRP3 inflammasome activation has been implicated in various inflammatory diseases, including pneumonia [21]. Recent studies have shown that the activation of NLRP3 inflammasome can be modulated by mitochondrial dysfunction, highlighting the interplay between mitochondria and inflammation [22,23].

Given the multifaceted roles of melatonin, telomerase, mitochondria, and NLRP3 inflammasome in the pathogenesis of pneumonia, it is intriguing to investigate their interrelationships and the potential therapeutic implications. This study aims to explore the effects of melatonin-induced upregulation of telomerase activity on mitochondrial metabolism and NLRP3 inflammasome activation in macrophages, a critical immune cell population involved in pneumonia pathogenesis. By elucidating the molecular mechanisms underlying the effects of melatonin on telomerase activity, mitochondrial function, and NLRP3 inflammasome activation in macrophages, we hope to shed light on the therapeutic potential of targeting these pathways for the treatment of pneumonia. Understanding how melatonin regulates these processes may not only provide insights into the interplay between mitochondria, telomeres, and inflammation but also reveal new avenues for developing effective therapies for pneumonia.

2. Methods

2.1. Establishment of Klebsiella pneumoniae-infected mouse model

Male C57BL/6J mice, 8 weeks old, weighing 20–23g, were purchased from the Experimental Animal Center, (Charles River, Beijing, China). The mice were randomly divided into two groups, with 10 mice in each group. Anesthesia was induced by intraperitoneal injection, and strict aseptic techniques were followed. Animals were anesthetized with pentobarbital sodium (30 mg/kg), administered by intraperitoneal injection. The neck was disinfected and the upper trachea of the mice was exposed. 1 ml syringe was used to puncture the trachea and inject 0.1 ml of Klebsiella pneumoniae solution (*K. pneumoniae* was obtained from the Biological Resource center of Tianjin First Central Hospital) [8]. Immediately after inoculation, the mice were placed in an upright position for approximately 20 s to ensure that the bacteria entered the lungs under the influence of gravity. The control group consisted of healthy mice that received no treatment, while the model group was established using the same method to induce Klebsiella pneumoniae-infected pneumonia in mice. The model group was treated with 2 mg/kg melatonin by oral gavage for 6 consecutive days after model establishment. Mice were euthanized by CO_2. The animal experiments met the ethical requirements and were approved by the Animal Care Ethics Committee of Tianjin First Central Hospital (2022-03-A1).

2.2. HE staining of lung tissue

Lung tissue samples were collected and fixed in 4 % paraformaldehyde (Beyotime, Shanghai, China). Routine dehydration, transparency, paraffin embedding, sectioning, and HE staining (Solarbio, Beijing, China) were performed to observe the pathological morphological changes in lung tissue.

2.3. Biochemical analysis of mouse blood

Mice were fasted for 12 h and venous blood samples were collected. After 1 h of standing, the serum was separated and stored at −20 °C. The biochemical parameters were measured using an automated biochemical analyzer (Thermo Fisher Scientific, USA). Total cell counts of neutrophils in different groups were detected.

2.4. Culture of bone marrow-derived macrophages (BMDMs)

Male C57BL/6 mice, 8 weeks old, were cervical dislocation and soaked in 75 % alcohol for 10 min for disinfection. The femurs and tibias of the mice were dissected, and the fur, muscles, and fascia were removed with forceps and scissors. The bones were soaked in sterile ice-cold PBS and then transferred to complete DMEM culture medium. The ends of the bones were cut off with a syringe, and the bone marrow cells were flushed out by blowing with a syringe. After collecting the cells with a filter, they were transferred to a 15 mL centrifuge tube and centrifuged at 1000 rpm for 5 min. The supernatant was discarded, and pre-warmed red blood cell lysis buffer was added to lyse the red blood cells. After 2 min of incubation, the cells were centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. Sterile PBS was added to resuspend the cells, and after centrifugation at 1000 rpm for 5 min, the supernatant was discarded. The cells were resuspended in complete DMEM culture medium (Hyclone, USA) containing 10 % FBS (Gibco, Life Technologies, Rockville, MD, USA). The cells were seeded in a 10 cm diameter cell culture dish at a density of 1×10^6/mL and incubated in a 37 °C, 5 % CO_2 incubator (Thermo Fisher Scientific, Waltham, MA, USA). After 24 h, the non-adherent cells were discarded, and the suspended cells were seeded in a 6-well plate. M-CSF was added to the culture medium at a concentration of 10 ng/mL. After three days, half of the medium was replaced, and M-CSF was added to maintain a concentration of 10 ng/mL. After three days, the adherent cells in the 6-well plate were collected to obtain mature bone marrow-derived macrophages (BMDMs). Mito-TEMPO was purchased from Enzo Life Sciences (Farmingdale, NY). Melatonin was purchased from Sigma-Aldrich (M5250, Sigma-Aldrich, St. Louis, MO, USA).

2.5. Cell transfection

Lipofectamine2000 Reagent (Life Technologies, Rockville, MD, USA) was diluted in 150 μl of optimized Opti-DMEM culture medium and incubated at room temperature for 5 min. The synthesized interfering plasmid (pcDNA3.1 vector (Invitrogen)) was dissolved in 700 μl of optimized Opti-DMEM culture medium to a final concentration of 14 μg. The diluted plasmid and Lipofectamine2000 Reagent were mixed and incubated at room temperature for 20–30 min. The mixture (250 μl) was added to each well of the corresponding 6-well plate. The cells were incubated at 37 °C, 5 % CO_2 for 24–48 h. After incubating for 48 h, the cells were digested with 0.05 % trypsin (Beyotime, Shanghai, China), and the cells were collected. After washing with DPBS three times, the cells were resuspended in 50 μl of RNA extraction lysis buffer or protein extraction lysis buffer. The cells were stored at −80 °C for RNA or protein extraction in the same background. NLRP3 siRNA sense (5′-GUUUGACUAUCUGUUCUdTdT-3′, GIMA Co. ,Shanghai, China).

2.6. qRT-PCR

Lung tissue or treated cells were used to extract total RNA using the TRIzol reagent kit (Thermo Fisher Scientific, Waltham, MA, USA). The RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The ReverTra Ace qPCR RT Master Mix kit (FSQ-301, TOYOBO (SHANGHAI) BIOTECH CO.,LTD.) was used to reverse transcribe the total RNA into cDNA in the reaction system (Applied Biosystem, CA, USA). The reaction system (25 μl) consisted of 0.5 μl upstream primer, 0.5 μl downstream primer, 1.0 μl cDNA, 12.5 μl Mix, and 10 μl sterile water. The reaction conditions were as follows: denaturation at 94 °C for 45 s, annealing at 59 °C for 45 s, and extension at 72 °C for 60 s, for a total of 35 cycles. Primers were synthesized by Sangon Bioengineering (Shanghai) Co., LTD. The change in gene expression levels was analyzed using the $2^{-\triangle\triangle Ct}$ method with GAPDH as the internal reference [24]. PCR sequences are provided in Table 1.

3. ELISA

Lung tissue homogenates were prepared from lung tissue samples, and the supernatant was collected after centrifugation. The levels of the inflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) (Thermo Fisher Scientific, Waltham, MA, USA) in the tissue homogenates or cells were measured using a sandwich ELISA kit. The kit was taken out from the refrigerator at 4 °C and allowed to stand at room temperature for 30 min. The experiment was performed strictly according to the instructions (incubation at 37 °C for 60 min after adding the enzyme-labeled solution, incubation at 37 °C for 15 min after adding the substrate), and the OD values of each well were read at 450 nm using an automated ELISA reader. The standard curve was plotted using CurveExpert1.3 analysis software, with the OD values as the ordinate and the concentration of the standard as the abscissa. The concentration of the measured substance in the sample was read from the standard curve based on the OD value.

Table 1
The PCR sequence used in this experiment.

	Forward	Reverse
NLRP3	5′-TCTTCTCAAGTCTAAGCACCAAC-3′	5′-ACAGCAATCTGATTCCAAAGTC-3′
Caspase-1	5′-TGGTCTTGTGACTTGGAGGA-3′	5′-TGGCTTCTTATTGGCACGAT-3′
IL-1β	5′-AGCCCATCCTCTGTGACTCA-3′	5′-TGTCGTTGCTTGGTTCTCCT-3′
TERC	5′-ACTGGTCTAGGACCCGAGAAG-3′	5′-TCAATGGTGCCTCTGGAGATT-3′
PGC-1α	5′-AAACTTGCTAGCGGTCCTCA-3′	5′-TGGCTGGTGCCAGTAAGAG-3′
TNFAIP3	5′-AACCAATGGTGATGGAAACTG-3′	5′-GTTGTCCCATTCGTCATTCC-3′
GAPDH	5′-AACTTTGGCATTGTGGAAGG-3′	5′-GGAGACAACCTGGTCCTCAG-3′

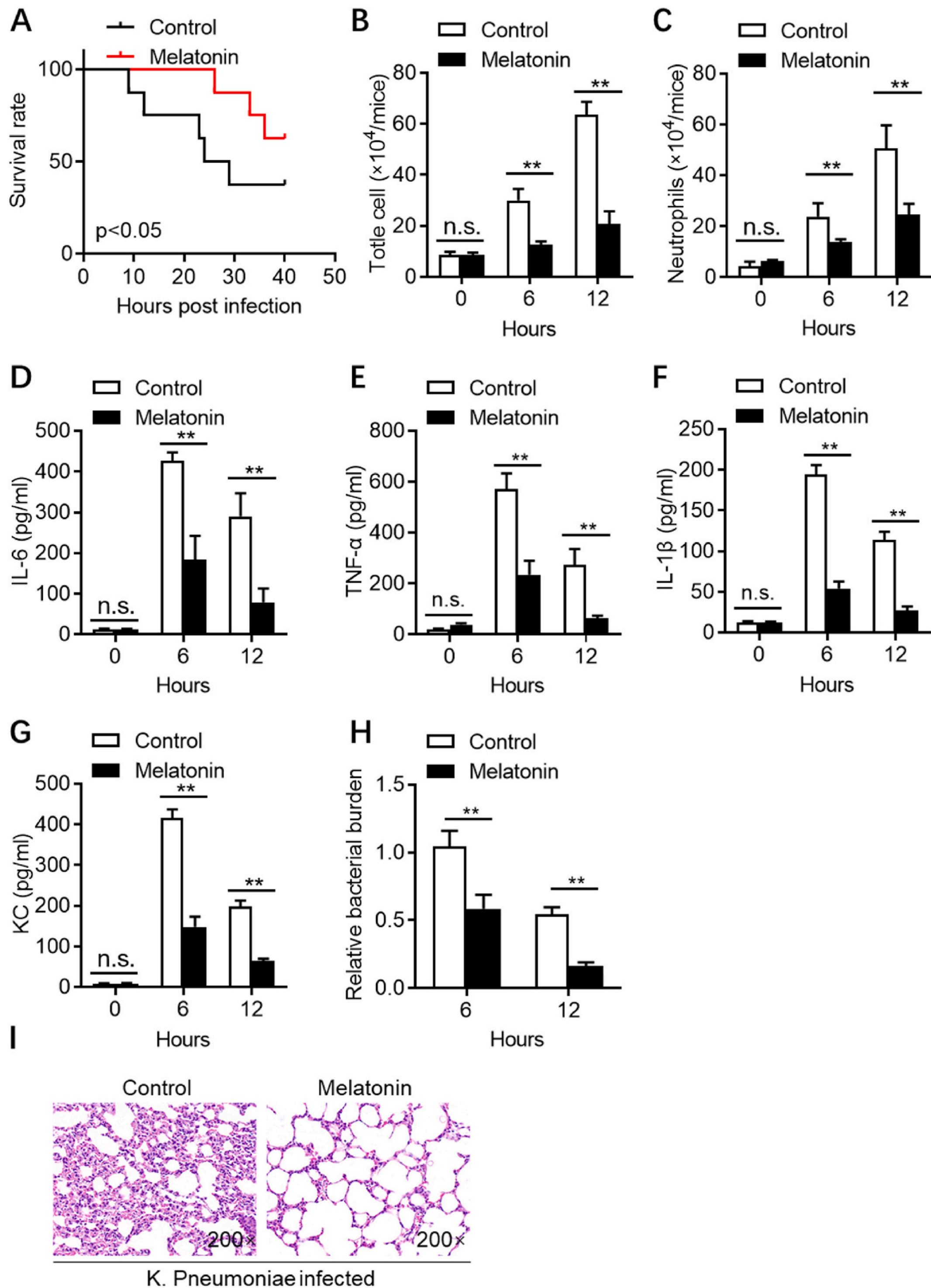

Fig. 1. Melatonin treatment reduces susceptibility to bacterial infection in mice. (A) Survival rate analysis of control and melatonin-treated mice (n = 10) injected with 10^8 colony-forming units (CFUs) of Klebsiella pneumoniae. Control and melatonin-treated mice (n = 10) were injected with 10^7 CFUs/mouse of Klebsiella pneumoniae and euthanized at specified time points. (B) Total cell count. (C) Neutrophil count. (D) Levels of IL-6 in bronchoalveolar lavage fluid (BALF). (E) Levels of TNF-α in BALF. (F) Levels of IL-1β in BALF. (G) Levels of KC in BALF. (H) Bacterial burden in BALF. (I) H&E staining of lung tissues K. Pneumoniae infected. N = 10, All results are expressed as the mean ± SD. ns: not significant, $^{**}p < 0.01$.

3.1. Western blot

Cell protein was extracted using a cell protein extraction kit according to the instructions. The protein concentration was determined using the BCA method. Thirty micrograms of protein was subjected to 10 % SDS-PAGE. The proteins were then transferred to a PVDF membrane (Millipore, Billerica, MA, USA), which was blocked with 5 % bovine serum albumin at room temperature for 2 h. The membrane was incubated with primary antibodies: IκBα (sc-371, SantaCruz, CA), caspase-1 (sc-514, SantaCruz, CA), p65 (8242S, Cell Signaling, Beverly, MA), *p*-IκBα (2859s, Cell Signaling, Beverly, MA), p-p65 (3039, Cell Signaling, Beverly, MA), Caspase1p20 (Adipogen, San Diego, CA) (1:1000), Anti-IL-1 beta antibody (ab315084, Abcam), Cleaved-IL-1 beta Antibody (#AF4006) overnight at 4 °C. After washing the membrane with TBST, it was incubated with horseradish peroxidase-conjugated anti-mouse IgG (1:10,000, Santa Cruz) at room temperature for 2 h. After washing the membrane with TBST, the enhanced chemiluminescence (ECL) (Millipore, catalog: MA01821) substrate kit was used for darkroom exposure.

3.2. Mito Tracker red labeling

Cells were washed with PBS twice and fixed with 4 % paraformaldehyde for 10 min. After washing with PBS for 10 min, 0.1 % Triton X-100 was added for permeabilization for 10 min, followed by washing with PBS for 10 min. The cells were placed on a glass slide and placed in a humid box. MitoTracker Deep Red (Life Technologies, Rockville, MD, USA) working solution was added to the cell slide and incubated at 37 °C for 30 min. After washing with PBS for 10 min, DAPI (Beyotime, Shanghai, China) (1 μg/mL) staining was added for 5 min, followed by washing with PBS for 10 min. The cells were MitoSox probe was added to each well at a final concentration of 5 μmol/mL to detect the level of mitochondrial ROS. The cells were incubated at 37 °C in the dark for 30 min. After fixation with 4 % paraformaldehyde at 37 °C for 15 min, the cells were incubated with DAPI (1 μg/mL) for 15 min and then treated with an anti-fluorescence quenching mounting agent. The cells were observed and photographed under a confocal microscope. ImageJ software (version 1.8.0) was used for analysis of the average fluorescence intensity.

3.3. JC-1 detection of mitochondrial membrane potential

The cells were treated as described above. After incubating for 24 h at 37 °C in a 5 % CO_2 incubator, the culture medium was removed, and the cells were washed with PBS once and then added with 0.5 mL of cell culture medium. Next, 0.5 mL of JC-1 staining working solution (Solarbio, Beijing, China) was added and mixed well. The cells were incubated at 37 °C in a 5 % CO_2 incubator for 20 min. After incubation, the supernatant was removed. The cells were washed twice with JC-1 staining buffer (1 ×) and then added with 1 mL of cell culture medium. The cells were observed and photographed under a fluorescence microscope (Nikon, Japan).

3.4. Statistical analysis

Data were analyzed using SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). Measurement data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used for comparison among multiple groups, and LSD-t test was used for pairwise comparison. Student's t-test was used for comparison between two groups. A p-value less than 0.05 was considered statistically significant.

4. Results

4.1. Melatonin treatment protects mice from bacterial infection

When mice in the control group and melatonin-treated group were injected with Klebsiella pneumoniae, it was observed that the survival rate of melatonin-treated mice was significantly higher than that of the control group. Specifically, the control group mice (n = 10) had a lower survival rate, while the melatonin-treated mice (n = 10) had a higher survival rate, indicating that melatonin treatment can significantly reduce the lethal effects of bacterial infection (Fig. 1A). Further analysis showed that the total cell count in the lungs of melatonin-treated mice was significantly lower than that in the control group. This indicates that melatonin treatment reduces the degree of inflammation and cell infiltration in the lungs (Fig. 1B). In addition, the neutrophil count in the melatonin-treated mice was also lower than that in the control group. Neutrophils are important inflammatory cells in the immune system, and a decrease in their number suggests that melatonin treatment suppresses the inflammatory response (Fig. 1C). Analysis of the levels of inflammatory cytokines in bronchoalveolar lavage fluid (BALF) showed that the levels of IL-6, TNF-α, IL-1β, and KC in the melatonin-treated mice were lower in the control group mice. This indicates that melatonin treatment can inhibit the release of inflammatory cytokines and reduce the inflammatory response (Fig. 1D–G). Furthermore, the bacterial load in the BALF of melatonin-treated mice was significantly lower in the control group. This suggests that melatonin treatment helps control the extent of bacterial infection and reduces bacterial replication and dissemination in the lungs (Fig. 1H). H&E staining of lung tissues K. Pneumoniae infected. The results showed that melatonin reduced the degree of pneumonia (Fig. 1I). Overall, these results indicate that melatonin treatment can inhibit the occurrence and development of pneumonia. Melatonin-treated mice showed higher survival rates, lower total cell counts, neutrophil counts, levels of inflammatory cytokines, and bacterial loads, indicating the potential anti-inflammatory and anti-infective effects of melatonin.

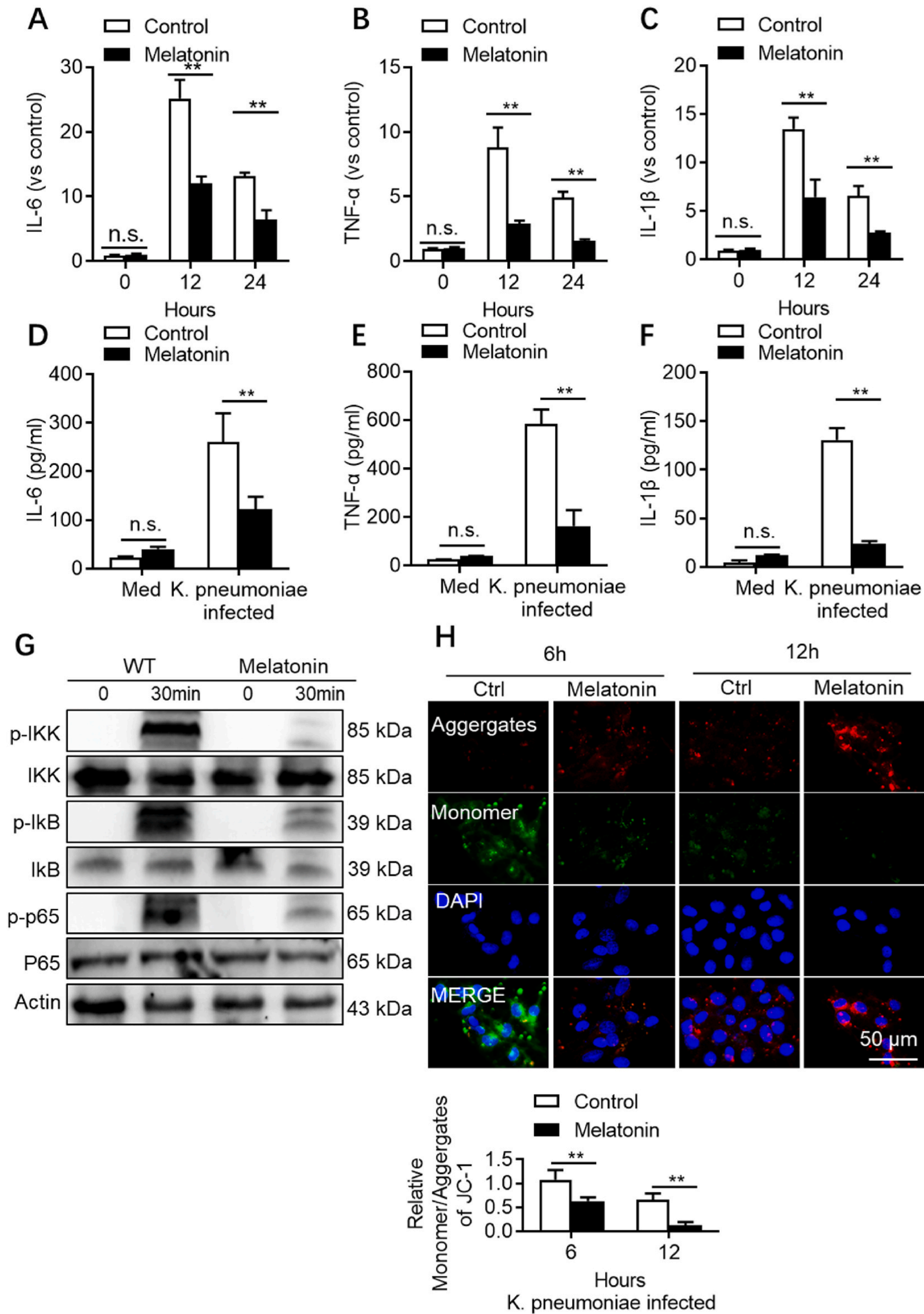

Fig. 2. Melatonin treatment reduces inflammation and antibacterial response in macrophages. Cell line: Bone marrow-derived macrophages (BMDMs) Under Klebsiella pneumoniae stimulation. (A–C) qRT-PCR analysis of TNF-α, IL-6, and IL-1β mRNA in melatonin-treated or control BMDMs. (D–F) ELISA analysis of TNF-α, IL-6, and IL-1β levels in melatonin-treated or control BMDMs. (G) Western blot analysis of total and phosphorylated proteins related to NF-κB in melatonin-treated or control BMDMs. (H) JC-1 probe analysis of mitochondrial membrane potential changes in melatonin-treated or control BMDMs. All results are expressed as the mean ± SD. ns: not significant, $^{**}p < 0.01$.

4.2. .Melatonin-treated macrophages exhibit reduced inflammatory and antibacterial responses

The results showed that melatonin-treated bone marrow-derived macrophages (BMDMs) exhibited reduced inflammatory and antibacterial responses under Klebsiella pneumoniae stimulation. In the experiment, BMDMs were divided into control group and melatonin treatment group. qRT-PCR analysis revealed that the mRNA levels of TNF-α, IL-6, and IL-1β in the melatonin treatment group were significantly lower than those in the control group. This indicates that melatonin treatment can inhibit the synthesis and release of inflammatory cytokines in BMDMs (Fig. 2A–C). Further analysis using ELISA showed that the levels of TNF-α, IL-6, and IL-1β in the melatonin treatment group were also significantly lower than those in the control group. This further confirms that melatonin treatment can reduce the levels of inflammatory cytokines in BMDMs (Fig. 2D–F). For the regulation of the NF-κB signaling pathway, Western blot analysis revealed that the levels of phosphorylated proteins related to NF-κB (*p*-IKK, *p*-IkB, and p-p65) in the melatonin treatment group were significantly lower than those in the control group, while the levels of total proteins (IKK, IkB, and P65) showed no significant difference. This indicates that melatonin treatment can inhibit the activation of the NF-κB signaling pathway, thereby reducing the synthesis and release of inflammatory cytokines (Fig. 2G & Supplementary fig. S1). Furthermore, JC-1 probe analysis showed that the changes in mitochondrial membrane potential in the melatonin treatment group BMDMs were significantly lower than those in the control group. This indicates that melatonin treatment can interfere with mitochondrial metabolism in BMDMs, thereby inhibiting the inflammatory and antibacterial responses (Fig. 2H). In summary, the results of the experiment indicate that melatonin treatment can reduce the synthesis and release of inflammatory cytokines in BMDMs, inhibit the activation of the NF-κB signaling pathway, and interfere with mitochondrial metabolism, thereby reducing the inflammatory and antibacterial responses.

4.3. Melatonin treatment suppresses excessive activation of NLRP3 inflammasome through upregulation of telomerase expression

First, the expression of telomerase in BMDMs was detected, and it was found that the expression of TERC in the melatonin treatment group BMDMs was significantly higher than that in the control group. This indicates that melatonin treatment can upregulate the expression of telomerase in BMDMs (Fig. 3A). Next, under Klebsiella pneumoniae stimulation, the levels of NLRP3, caspase-1, IL-1β and Cleaved IL-1β in the lysates of BMDMs were detected, and it was found that the levels of NLRP3, caspase-1, IL-1β and Cleaved IL-1β in the melatonin treatment group BMDMs were significantly lower than those in the control group. This indicates that melatonin treatment can suppress the excessive activation of NLRP3 inflammasome in BMDMs, thereby reducing the synthesis and release of

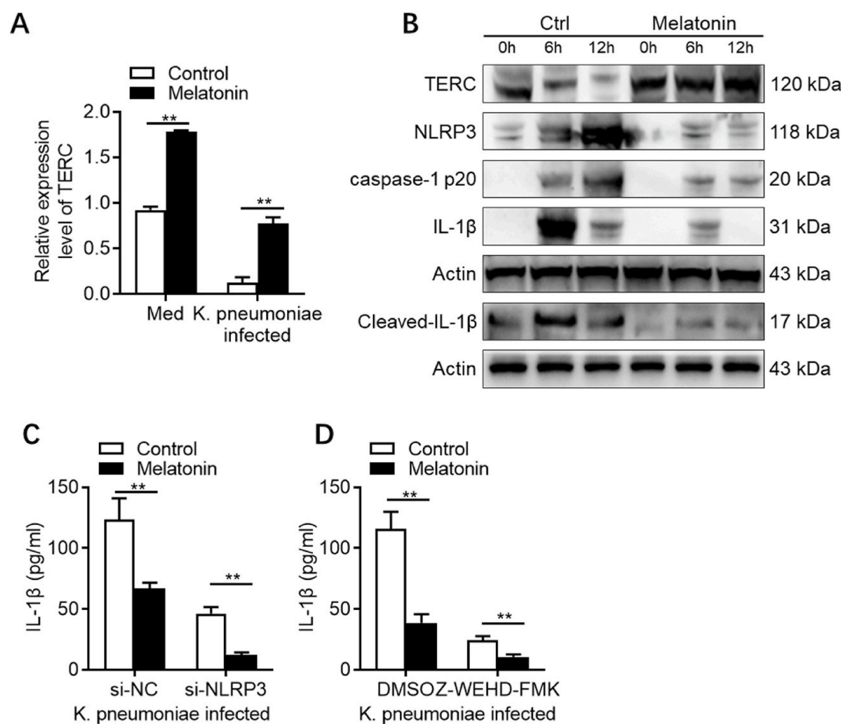

Fig. 3. Melatonin treatment inhibits excessive activation of NLRP3 inflammasome by upregulating telomerase expression. (A) Telomerase expression analysis in melatonin-treated or control BMDMs. (B) Levels of NLRP3, caspase-1, IL-1β and Cleaved-IL-1β in cell lysates (Lys) of melatonin-treated or control BMDMs infected with Klebsiella pneumoniae at specified time points. (C) Melatonin-treated or control BMDMs transfected with non-specific control (NC) or NLRP3-targeting siRNA, followed by infection with bacteria for 12 h and detection of IL-1β secretion. (D) Melatonin-treated or control BMDMs treated with Z-WEHD-FMK, followed by infection with bacteria for 12 h and detection of IL-1β secretion. All results are expressed as the mean ± SD. ns: not significant, $^{**}p < 0.01$.

inflammatory cytokines (Fig. 3B & Supplementary fig. S2). To further validate the inhibition of NLRP3 inflammasome activation by melatonin treatment, the following experiments were performed: First, BMDMs were transfected with non-specific control (NC) or NLRP3-targeting siRNA and divided into four groups: control group NC, control group siRNA, melatonin treatment group NC, and melatonin treatment group siRNA. After 12 h of Klebsiella pneumoniae infection, IL-1β secretion was detected. The results showed that the secretion of IL-1β in the melatonin treatment group NC and control group siRNA was significantly lower than that in the control group NC. This indicates that melatonin treatment can reduce the synthesis and release of inflammatory cytokines by inhibiting the excessive activation of NLRP3 inflammasome (Fig. 3C). Second, BMDMs were treated with Z-WEHD-FMK and divided into melatonin treatment group and control group. After 12 h of Klebsiella pneumoniae infection, IL-1β secretion was detected. The results showed that the secretion of IL-1β in the melatonin treatment group was significantly lower than that in the control group. This indicates that melatonin treatment can reduce the synthesis and release of inflammatory cytokines by inhibiting the activity of caspase-1 (Fig. 3D). In conclusion, the experimental results indicate that melatonin treatment can upregulate the expression of telomerase in BMDMs, suppress the excessive activation of NLRP3 inflammasome, and reduce the synthesis and release of inflammatory cytokines.

4.4. Silencing TERC reduces the lung-protective effect of melatonin

In the experiment, BMDMs were divided into four groups: siRNA NC group (control group), siRNA NC + melatonin group (control group with melatonin treatment), si-TERC group (silencing TERC), and si-TERC + melatonin group (silencing TERC with melatonin treatment). The expression levels of NLRP3, caspase-1, and IL-1β were detected to evaluate the extent of the inflammatory response. For the expression levels of NLRP3, caspase-1, and IL-1β (Fig. 4A–C), the following trends were observed: the expression levels were lowest in the siRNA NC + melatonin group, indicating that melatonin treatment can suppress the excessive activation of NLRP3 inflammasome, reduce the activity of caspase-1, and inhibit the synthesis and release of IL-1β; the expression levels were higher in the siRNA NC group, indicating that without melatonin treatment, the expression levels of NLRP3, caspase-1, and IL-1β were relatively higher; the expression levels were similar in the si-TERC group and si-TERC + melatonin group, suggesting that silencing TERC has no significant effect on the lung-protective effect of melatonin. In addition, for the levels of IL-1β, TNF-α, and IL-6 (Fig. 4D–F), similar trends were observed: the levels were lowest in the siRNA NC + melatonin group, indicating that melatonin treatment can reduce the synthesis and release of IL-1β, TNF-α, and IL-6; the levels were higher in the siRNA NC group, indicating that without melatonin treatment, the levels of inflammatory cytokines were relatively higher; the levels were similar in the si-TERC group and si-TERC +

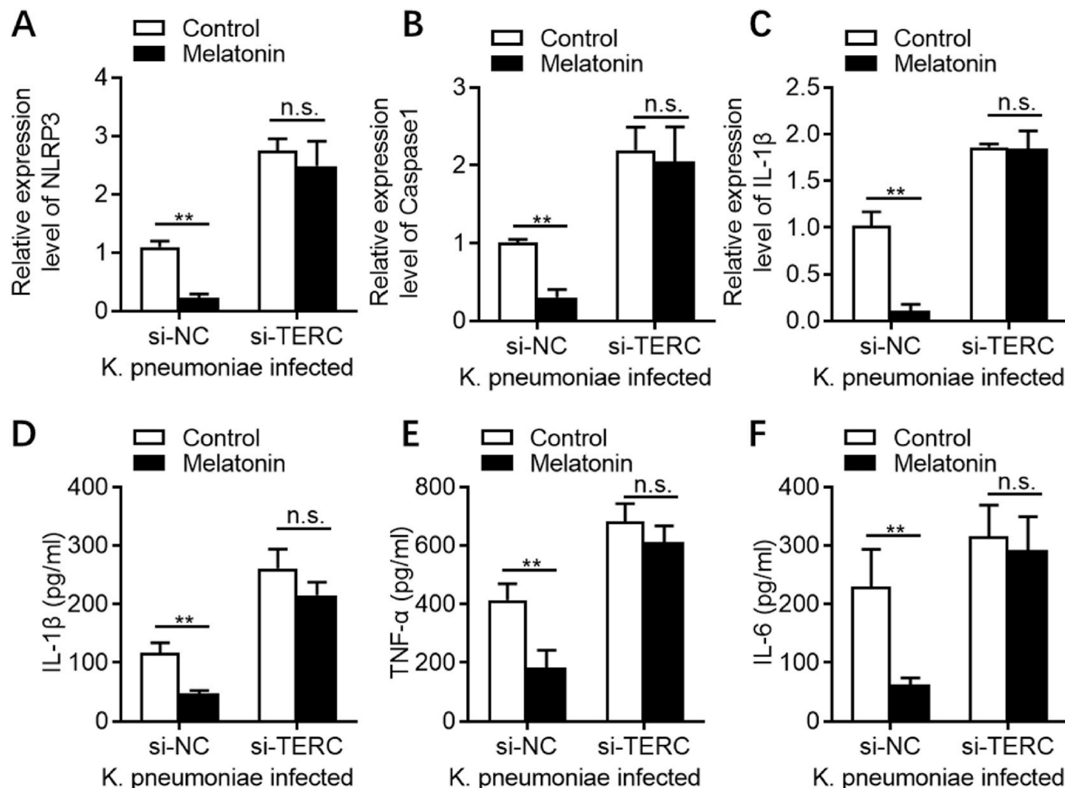

Fig. 4. Silencing TERC reduces the protective effect of melatonin in pneumonia. (A–C) Expression analysis of NLRP3, Caspase1, and IL-1β in melatonin-treated or control BMDMs with TERC silencing. (D–F) Levels of IL-1β, TNF-α, and IL-6 in melatonin-treated or control BMDMs with TERC silencing. All results are expressed as the mean ± SD. ns: not significant, **$p < 0.01$.

melatonin group, suggesting that silencing TERC has no significant effect on the lung-protective effect of melatonin. In conclusion, the experimental results indicate that silencing TERC reduces the lung-protective effect of melatonin, leading to excessive activation of NLRP3 inflammasome and increased synthesis and release of inflammatory cytokines.

4.5. Melatonin protects against mitochondrial abnormalities and oxidative stress, reducing inflammasome activation

For the assessment of mitochondrial function, BMDMs were stained with MitoTracker Red to visualization the mitochondria. The results showed that the fluorescence intensity of mitochondria was lower in the control group, indicating impaired mitochondrial function, while it was higher in the melatonin treatment group, indicating better mitochondrial function (Fig. 5A). Therefore, it can be concluded that melatonin treatment can improve mitochondrial function. In addition, ATP levels were measured in the cells, and it was observed that ATP levels were lower in the control group and higher in the melatonin treatment group. This indicates that melatonin treatment can increase cellular ATP levels (Fig. 5B). In terms of oxidative stress, BMDMs were stained with Mito-SOX to assess mitochondrial oxidative stress levels. The results showed that the melatonin treatment group had lower levels of oxidative stress compared to the control group. This indicates that melatonin treatment can reduce mitochondrial oxidative stress (Fig. 5C).

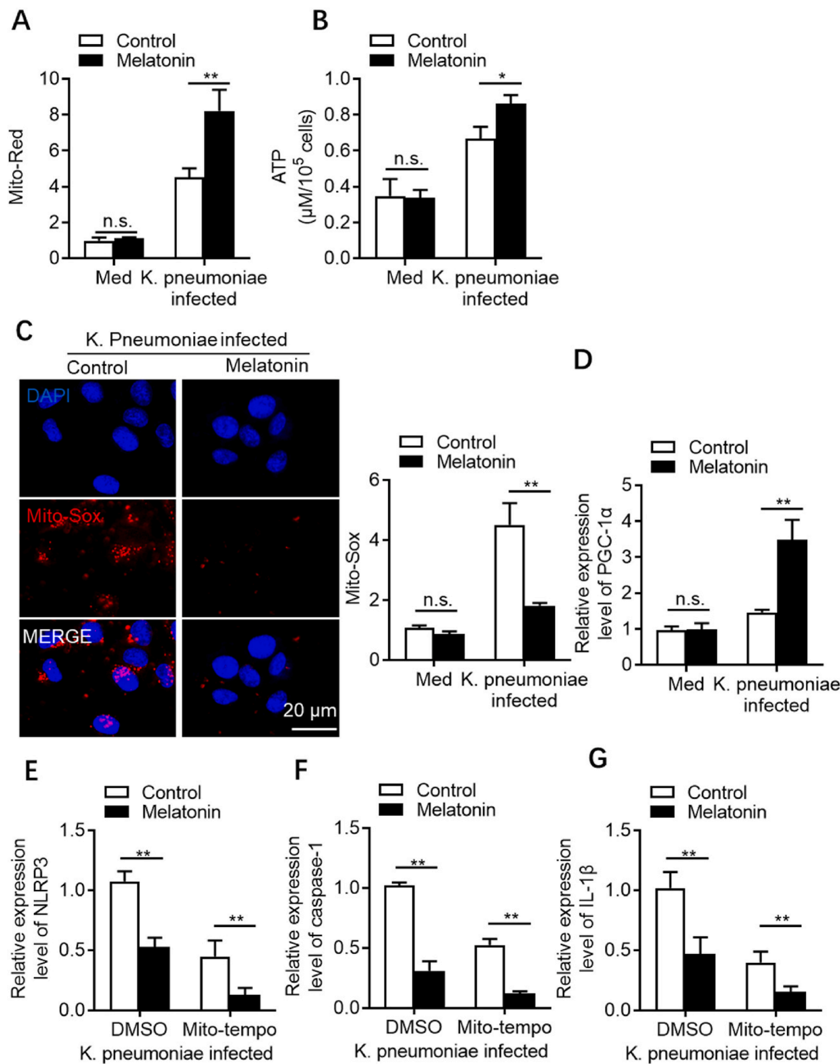

Fig. 5. Melatonin protects against mitochondrial dysfunction and oxidative stress, reducing inflammasome activation. Melatonin-treated and control BMDMs infected with Klebsiella pneumoniae for 12 h. (A) Mitochondrial detection in BMDMs stained with MitoTracker Green. (B) ATP levels in cells. (C) Detection of BMDMs labeled with Mito-SOX. (D) qRT-PCR analysis of PGC-1α levels in melatonin-treated and control BMDMs infected with Klebsiella pneumoniae at specified time points. (E) mRNA levels of NLRP3 in melatonin-treated or control BMDMs pre-treated with DMSO or Mito-tempo, followed by infection with bacteria. (F) mRNA levels of caspase-1 in melatonin-treated or control BMDMs pre-treated with DMSO or Mito-tempo, followed by infection with bacteria. (G) mRNA levels of IL-1β in melatonin-treated or control BMDMs pre-treated with DMSO or Mito-tempo, followed by infection with bacteria. All results are expressed as the mean ± SD. ns: not significant, $*p < 0.05$, $**p < 0.01$.

Furthermore, melatonin treatment increased the expression level of PGC-1α, further enhancing mitochondrial function (Fig. 5D). Finally, BMDMs were pretreated with DMSO or Mito-tempo and divided into melatonin treatment or control group. After Klebsiella pneumoniae infection, the levels of NLRP3, caspase-1, and IL-1β were measured (Fig. 5E–G). The results showed that the melatonin treatment group had lower mRNA levels of NLRP3, caspase-1, and IL-1β compared to the control group. This indicates that melatonin treatment can reduce the formation of inflammasomes. These results suggest that melatonin has anti-inflammatory effects and can reduce inflammasome formation by protecting against mitochondrial dysfunction and oxidative stress.

4.6. TNFAIP3 is involved in the inflammatory pathology regulated by melatonin

Different markers were used to evaluate the effects of melatonin on the inflammatory pathology. First, the mRNA level of TNFAIP3 was measured. TNFAIP3 is an important anti-inflammatory factor that can inhibit inflammation and regulate inflammatory signaling pathways. The results showed that the melatonin treatment group had significantly higher TNFAIP3 mRNA levels compared to the control group. This indicates that melatonin treatment can increase the expression of TNFAIP3, thereby suppressing the inflammation (Fig. 6A). Silencing TNFAIP3 reduced the lung-protective effect of melatonin and led to a significant increase in IL-1β expression. This suggests that TNFAIP3 plays an important role in the anti-inflammatory effects regulated by melatonin. Melatonin treatment can increase the expression of TNFAIP3, thereby suppressing inflammation. Silencing TNFAIP3 reduces the lung-protective effect of melatonin, indicating a weakened anti-inflammatory effect of melatonin and an increase in IL-1β expression, exacerbating the inflammatory response (Fig. 6B). In conclusion, the experimental results indicate that melatonin treatment can increase the expression of TNFAIP3, thereby suppressing inflammation. Silencing TNFAIP3 reduces the lung-protective effect of melatonin and leads to increased IL-1β expression.

4.7. The proposed mechanism of action of melatonin in interfering with immune and metabolic balance during the host defense response

Melatonin treatment inhibits the production and release of pro-inflammatory cytokines, reducing inflammation and immune cell infiltration. Additionally, melatonin enhances antioxidant and anti-stress capabilities, protecting cells from oxidative damage and apoptosis. These mechanisms help maintain immune and metabolic balance in the host, thereby improving resistance to infection and stimuli (Fig. 7). In summary, the findings suggest that melatonin treatment can suppress inflammation and enhance host defense against bacterial infection. Melatonin exerts its protective effects by reducing inflammation, inhibiting NLRP3 inflammasome activation, improving mitochondrial function, and upregulating TERT expression. These results provide insights into the potential therapeutic applications of melatonin in the treatment of inflammatory diseases.

5. Discussion

Pneumonia is a common respiratory infection characterized by inflammation of the lungs, and it remains a significant cause of morbidity and mortality worldwide [5]. The search for effective therapeutic strategies to combat pneumonia is ongoing, and recent studies have shed light on the potential benefits of melatonin in the treatment of this respiratory infection [25].

Klebsiella pneumoniae is a gram-negative bacterium that can enter the lungs through the respiratory tract [7]. When Klebsiella pneumoniae grows and multiplies in the alveoli, it can cause congestion of alveolar capillaries, edema and inflammatory cell infiltration [26]. In this study, a mouse model of Klebsiella pneumoniae infection was established by endotracheal drip method. In our

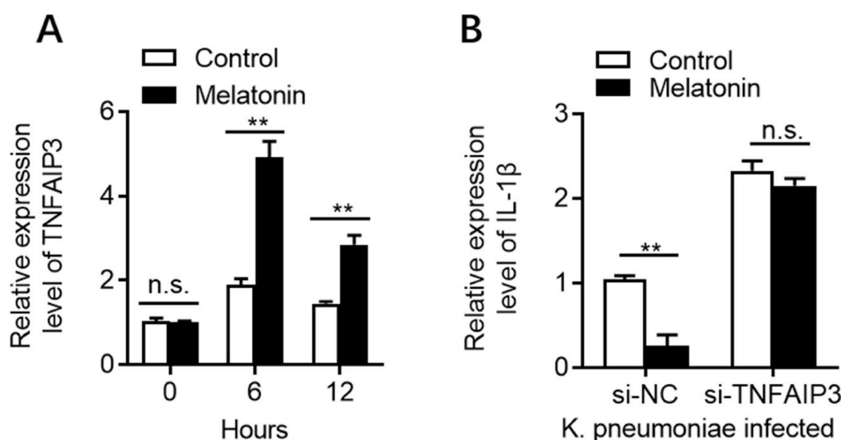

Fig. 6. TNFAIP3 is involved in the inflammatory pathology regulated by melatonin. Melatonin-treated and control BMDMs infected with Klebsiella pneumoniae for 12 h. (A) mRNA levels of TNFAIP3 in melatonin-treated and control BMDMs infected with Klebsiella pneumoniae at specified time points. (B) Expression analysis of IL-1β in melatonin-treated or control BMDMs with TNFAIP3 silencing. All results are expressed as the mean ± SD. ns: not significant, $^{**}p < 0.01$.

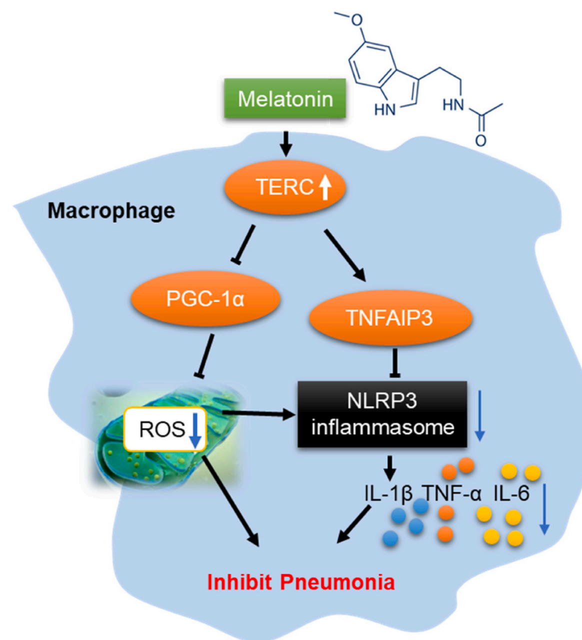

Fig. 7. Melatonin targets TERC to interfere with immune and metabolic balance during host defense response.

study, we investigated the effects of melatonin-induced upregulation of telomerase activity and the PGC-1α/TNFAIP3 axis on macrophage mitochondrial metabolism and NLRP3 inflammasome activation [27]. Our findings revealed several key observations that contribute to our understanding of the underlying mechanisms and potential therapeutic implications [28].

First, we demonstrated that melatonin treatment significantly upregulates telomerase activity in macrophages. Telomerase is an enzyme responsible for maintaining the length of telomeres, which are protective structures at the ends of chromosomes. Telomeres play a crucial role in maintaining genomic stability, and their shortening is associated with cellular senescence and dysfunction. By upregulating telomerase activity, melatonin may help preserve telomere length, thereby promoting cellular health and function in macrophages.

Furthermore, melatonin treatment was found to interfere with macrophage mitochondrial metabolism through the activation of the PGC-1α/TNFAIP3 axis [29]. PGC-1α is a key regulator of mitochondrial biogenesis and function, while TNFAIP3 is an anti-inflammatory protein that inhibits the activation of pro-inflammatory pathways [30]. Our study demonstrated that melatonin treatment upregulates the expression of PGC-1α and TNFAIP3 in macrophages, suggesting enhanced mitochondrial function and reduced inflammation. This suggests that melatonin may confer protection against mitochondrial damage and dysfunction, mitigating the inflammatory response in pneumonia.

The NLRP3 inflammasome is a key component of the innate immune system and plays a significant role in the initiation and regulation of inflammatory responses [31,32]. In our study, we found that melatonin treatment dampened NLRP3 inflammasome activation in macrophages. The NLRP3 inflammasome is involved in the production of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) [33,34]. Our results showed reduced secretion of IL-1β upon melatonin treatment, indicating the inhibition of NLRP3 inflammasome activation. This anti-inflammatory effect of melatonin on the NLRP3 inflammasome suggests its potential to modulate immune responses in pneumonia.

Collectively, our findings suggest that melatonin-induced upregulation of telomerase activity and the PGC-1α/TNFAIP3 axis interferes with macrophage mitochondrial metabolism and suppresses NLRP3 inflammasome activation, offering a promising approach for pneumonia treatment. By promoting telomere maintenance and activating the PGC-1α/TNFAIP3 axis, melatonin may help preserve macrophage function, enhance mitochondrial function, and attenuate the inflammatory damage observed in pneumonia.

These findings highlight the potential therapeutic role of melatonin in the management of pneumonia [35]. The modulation of telomerase activity, the PGC-1α/TNFAIP3 axis, and NLRP3 inflammasome activation by melatonin provides a multifaceted approach to alleviate the inflammatory response, enhance immune function, and ultimately improve patient outcomes in pneumonia.

However, it is important to acknowledge some limitations of our study. Firstly, our experiments were conducted in vitro using macrophage cell lines, which may not fully reflect the complexity of the in vivo environment. Hence, further studies using animal models and clinical trials are warranted to validate our findings. Secondly, although our study provides insight into the underlying mechanisms, the specific molecular pathways through which melatonin upregulates telomerase activity, activates the PGC-1α/TNFAIP3 axis, and modulates NLRP3 inflammasome activation require further investigation.

6. Conclusion

In conclusion, our study demonstrates that melatonin-induced upregulation of telomerase activity and the PGC-1α/TNFAIP3 axis interferes with macrophage mitochondrial metabolism and suppresses NLRP3 inflammasome activation, offering a multifaceted approach for the treatment of pneumonia. These findings provide a rationale for exploring the therapeutic potential of melatonin in pneumonia management and suggest avenues for further research in the field. Ultimately, the development of novel therapeutic strategies targeting these molecular pathways may hold promise for improving outcomes in pneumonia patients.

Ethics approval and consent to participate

The animal experiments met the ethical requirements and were approved by the Animal Care Ethics Committee of Tianjin First Central Hospital (2022-03-A1).

Consent for publication

Not applicable.

Data availability statement

Data included in article/supp. material/referenced in article.

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CRediT authorship contribution statement

Wei Jiang: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Jun Liu:** Writing – original draft, Formal analysis, Data curation. **Xuequn Zhao:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Wenjie Yang:** Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29681.

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