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MicroRNA-494 inhibition alleviates acute lung injury through Nrf2 signaling pathway via NQO1 in sepsis-associated acute respiratory distress syndrome

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ABSTRACT

Aims: Although therapeutic strategies for acute respiratory distress syndrome (ARDS) have achieved improvements, its mortality remains high. It has been reported that microRNAs (miRs) serve as therapeutic strategies for ARDS, while specific mechanisms of miR-494 remain poorly understood. Thus, the present study aimed to assess the effects of miR-494 on acute lung injury (ALI) in rat models of sepsis-associated ARDS and its regulatory mechanism.

Methods: Following establishment of sepsis-associated ARDS rat models, the ratio of wet to dry weight (W/D) in right lung tissues was detected. Moreover, the expression patterns of miR-494, NQO1 and Nrf2 were evaluated in left lung tissues of rats. The miR-494 expression was overexpressed in rats so as to analyze the effects of miR-494 on ALI, inflammatory response and oxidative stress. Meanwhile, the Nrf2 signaling pathway was activated in rats in order to show the regulatory mechanism of miR-494 in ALI. And the target gene of miR-494 was identified by dual-luciferase reporter assay.

Key findings: The findings firstly revealed upregulated miR-494, and enhanced inflammatory response, oxidative stress and ALI in rats with sepsis-associated ARDS. Additionally, MiR-494 negatively regulated NQO1 and Nrf2 expression level. Moreover, ectopic expression of miR-494 promoted inflammatory response, oxidative stress and ALI. However, the activation of Nrf2 signaling pathway reversed these effects of miR-494.

Conclusion: Our findings highlight the value of miR-494 inhibition as a therapeutic target for sepsis-associated ARDS.

1. Introduction

Acute respiratory distress syndrome (ARDS) is a life-threatening form of respiratory failure that results from a primary disease of pneumonia, aspiration pneumonia, extrathoracic sepsis, etc. [1,2]. The main characteristic of ARDS pathogenesis is systemic inflammation that leads to alveolar damage, as well as the exudation of pulmonary edema fluid with protein accumulation in the alveolar space, resulting in respiratory distress [3]. Although therapeutic strategies for ARDS have achieved improvements, ARDS remains substantial mortality in critically ill patients, especially sepsis-associated [4,5]. Accumulating evidence has indicated that statins, such as rosuvastatin, is regarded as the most common strategy for sepsis-associated ARDS, while it shows impairments both physically and mentally, including toxicity to liver and skeletal muscle, together with incident post-traumatic stress disorder (PTSD) symptoms related to nightmares, paranoia, and depression [6]. In recent years, microRNAs (miRs) have been suggested as diagnostic biomarkers or therapeutic targets in ARDS [7]. MiRs refer to small non-coding RNA molecules, which serve as regulators in inhibition of messenger RNA (mRNA) translation or reduction of mRNA stability by binding the 3’ untranslated region (3’UTR) of target mRNA [8,9]. It has also been shown that miRs exert great positive effects on lung and systemic inflammation [10]. Moreover, dysregulated expressions of several miRs are involved in the ARDS pathogenesis, such as miR-21, miR-146, miR-17, etc. [11]. Therefore, a new miR-based therapeutic target is of great importance for sepsis-associated ARDS.

MiR-494, located on chromosome 14q32.31, is upregulated in lung cancer cells and serves as the therapeutic targets for lung cancer [12]. It
not only participates in the pathogenesis of acute kidney injury (AKI) [13], but also plays a vital role in ischemia-induced injury [14]. It is reported that miR-494 promotes glioma cell invasion by regulating the expression of its target gene [15], while the specific mechanisms of miR-494 involving sepsis-associated ARDS remain poorly understood. Thus, the aim of the present study was to investigate the roles of miR-494 in sepsis-associated ARDS, with the ARDS rat model construction. NAD(P)H:quinone oxidoreductase 1 (NQO1), a phase II/antioxidant enzyme, also participates in acute lung injury (ALI), which is a form of ARDS, by modulating the formation of reactive oxygen species (ROS) and other free radicals [7,16]. In addition, NQO1 expression can be upregulated by nuclear factor E2 p45-related factor 2 (Nrf2) by binding to antioxidant response elements (ARE) [17]. Nrf2, a basic-region leucine zipper transcription factor, acts as a regulator in lung inflammation by modulating anti-oxidant and cytokine gene expression [18,19]. Furthermore, Mehla et al. have implied that activation of Nrf2 provides potential benefits for protection against ALI [19]. Therefore, the present study was to explore modulatory effects of miR-494 on the ALI, and the regulatory mechanism. Based on the aforementioned literature, we proposed a hypothesis that miR-494 may enhance ALI by targeting NQO1 through Nrf2 signaling pathway in sepsis-associated ARDS rats.

2. Materials and methods

2.1. Ethics statement

All animal experiments conducted during this study were in strict adherence with the Guide for the Care and Use of Laboratory Animals by US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All experimental procedures involving rats were approved by local ethics Committee of the Second Affiliated Hospital of Guangxi Medical University (Certificate Number: 201706004).

2.2. Grouping of experimental animals and establishment of sepsis-associated ARDS model

A total of 40 healthy male Sprague-Dawley (SD) rats (age, 8–9 weeks, weighing 240–280 g) were purchased from Experimental Animal Research Center. All rats were housed in a controlled specific pathogen free (SPF) facility environment at 24 °C with a relative humidity of 50%, alternating light and dark periods for 12 h each.

In accordance with the random number table, all rats were assigned into the sham group (sham-treated rats), ALI group (ALI rats without treatment), mimic-NC group (ALI rats treated with short interfering RNAs [siRNAs] with same batch number of miR-494 and irrelevant with existed miRs, mimics-NC), miR-494 mimic group (ALI rats treated with miR-494 mimics), miR-494 mimic + SFN group (ALI rats treated with miR-494 mimics + SFN), mimic-NC group (ALI rats treated with short interfering RNA with same batch number of miR-494 and irrelevant with existed miRs, mimics-NC), miR-494 mimic group (ALI rats treated with miR-494 mimics) and miR-494 mimic + SFN group (ALI rats treated with miR-494 mimics + SFN) groups (n = 8). All rats were fasted for 12 h before the experiment. Next, rats were anesthetized, and fixed on the operating table, followed by abdominal hair removal. The abdomen of rats was opened, and the cecum of rats in the sham group was pulled out and sutured. With an exposure of the abdomen of rats, the cecum of rats in the four groups was ligated with silk thread in the middle of cecum. The puncture needle was used to puncture cecum from the mesenteric side to the opposite side. The appropriate amount of intestinal contents was squeezed out, and then the cecum was sutured. The treatment of miR-494 mimic or microRNA mimic NC was conducted at 1 h before surgery. The rats were anesthetized, and fixed on the operating table, followed by neck hair removal. Next, the skin was open, and organ was exposed. The transection complex (50 μL) was instilled into the trachea with micro-syringe. After transection, rats were erected and rotated so that transection complex was evenly distributed in lung. Rats in the miR-494 mimic + SFN group were intraperitoneally injected with SFN (5 mg/kg) at 30 min before model establishment. The miR mimic NC and miR-494 mimic were purchased from Sigma-Aldrich Chemical Company (St Louis MO, USA), and SFN from Shanghai HuZheng Biotechnology Co., Ltd. (Shanghai, China).

2.3. Animal treatment

At 24 h after surgery, rats were sacrificed, and chest of rats was opened and lung of rats was ligated. After lung lavage, bronchoalveolar lavage fluid (BALF) was collected. Some part of left lobe of the lung was preserved at −80 °C for extraction of total protein and RNA, and the other part of left lobe of the lung was immersed in 10% formaldehyde for histological detection and immunohistochemistry assay. The middle lobe of the right lung was resected, and the dry weight of which was weighed. Next, it was dried in thermostat at 37 °C for 72 h and weighed again, which was recorded as dry weight. The ratio of wet to dry weight (W/D) was calculated.

2.4. Hematoxylin-eosin (HE) staining

After fixation for 24 h, lung tissues were dehydrated and cleared with xylene, followed by paraffin embedding. The paraffin-embedded sections were sliced, dried, and stained. Subsequently, the sections were treated with HE staining. Sections were stained using hematoxylin for 5 min and rinsed with distilled water, followed by color separation with alcohol hydrochloric acid. Next, the samples were stained with eosin for 2 min, dehydrated, cleared, and mounted. Histological changes were observed and photographed with an optical microscope.

2.5. Immunohistochemistry assay

After phosphate buffer saline (PBS)-rinses, the sections of left lung tissues were blocked with 3% H2O2 for 15 min, and with 1% bovine serum albumin (BSA) for 30 min. Next, the sections were rinsed, and added with the primary antibody NQO1 (1:50, Abcam Inc., Cambridge, MA, USA) for incubation at 37 °C for 2 h. After rinses, the sections were incubated with biotin-labeled secondary antibody (Abcam Inc., Cambridge, MA, USA) at 37 °C for 2 h. The sections were rinsed again, and incubated with avidin-biotin peroxidase complex (ABC) at 37 °C for 1 h. Next, the sections were rinsed, developed, dehydrated, cleared, and mounted. Five fields (200×) were randomly selected to calculate the percentage of positive cells. Semi-quantitative analysis of NQO1 expression was conducted according to the staining intensity. The positive expression of NQO1 was presented as brown particles. The cells with brown particles of ≥5% were regarded to be positive, and cells with brown particles of < 5% were regarded to be negative [20].

2.6. Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Left lung tissues from rats in each group were collected, and total RNA was extracted from the left lung tissues with a Trizol kit (Invitrogen Inc., Carlsbad, CA, USA). The spectrophotometer (Bio-Rad Inc., Hercules, CA, USA) was employed to measure the ratio of D260/D280 in RNA and the concentration of RNA. A ratio of D260/D280 ranged 1.8–2.0 was identified as high purity of extracted RNA, which was used for further test. The reagent kits provided by TianGen Biotech Co., Ltd., (Beijing, China) were used for detection of miRNA sequence. The other primer sequences for PCR were synthesized by Shanghai Sangon Biotech Co., Ltd., (Shanghai, China). The primer sequences are shown in Table 1. The reverse transcription of RNA into cDNA and preparation of qPCR system were conducted in accordance with the instructions of cDNA reverse transcription kit (Takara Holdings Inc., Kyoto, Japan) and qPCR reagent kit (TianGen Biotech Co., Ltd., Beijing, China), respectively. The reaction was performed using a real-time PCR instrument (Applied Biosystems, Foster City, CA, USA). Reaction conditions were as follows: pre-denaturation at 95 °C for 15 min, 40 cycles...
Table 1
Primer sequences of related genes for reverse transcription quantitative polymerase chain reaction.

| Gene   | Sequence (3′-5′)          |
|--------|---------------------------|
| miR-494| F: TCACCCTACTTTGCTCCAACAA |
| NQO1   | F: TTTCCGCCTCCCTGAAATCATC |
| SOD    | F: CCAGGAAACACAGATGACG    |
| CAT    | F: AAGAGGAGGCCCCTTCTTCA   |
| GAPDH  | F: TCCCTCAATTTTGTCGCAAG   |
|        | R: AAGGGATGAAGGTGAGATGATC |

Note: miR-494, microRNA-494; NQO1, NAD(P)H:quinone oxidoreductase 1; SOD, superoxide dismutase; HO-1, heme oxygenase-1; CAT, catalase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; F, forward; R, reverse.

denaturation at 95 °C for 10 s, annealing and extension at 60–66 °C for 20–32 s. The miR-494 level and mRNA levels of NQO1, superoxide dismutase (SOD), and heme oxygenase-1 (HO-1) were detected.

2.7. Western blot analysis

Protein was extracted from the left lung tissues of rats in each group and the concentration was measured using bicinchoninic acid (BCA) kit (Wuhan Boster Biological Technology Ltd., Hubei, China). The extracted protein (30 μg/well) was added with the loading buffer and boiled at 95 °C for 5 min, and separated on 10% polyacrylamide gels (Wuhan Boster Biological Technology Co., Ltd., Hubei, China) with voltage of electrophoresis changing from 80 V into 120 V. Subsequently, the samples were transferred onto a polyvinylidene fluoride (PVDF) membrane using the wet transfer method at 100 mv for 70 min. Then, the membranes were blocked in 10% skimmed milk for 1 h at room temperature. Samples were incubated at 4 °C overnight with the primary antibodies NQO1 (1:500, Abcam Inc., Cambridge, MA, USA), Nrfl2 (1:1000, Abcam Inc., Cambridge, MA, USA), β-actin (1:1000, Santa Cruz Biotechnology, Inc., CA, USA). The membranes were washed with tris-buffered saline and Tween 20 (TBST) 10 times, 5 min at each, and cultured with the secondary antibody IgG-alkaline phosphatase antibody labeled with horsemen peroxidase (ab20272, Abcam Inc., Cambridge, MA, USA), 37 °C for 2 h and rinsed with TBST for 3 times, 5 min for each. The membrane was developed by diaminobenzidine (DAB). The gray value ratio of target protein band was analyzed using Image J software.

2.8. Enzyme-linked immunosorbent assay (ELISA)

The BALF in each group was collected and centrifuged at 12000 × g for 10 min. The supernatant was collected and the levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α were detected using ELISA kit. The test for each sample was conducted with at least 3 parallel wells. ELISA kits for TNF-α, IL-1β, and IL-6 were purchased fromCUSABIO Biotech Co., Ltd. (Wuhan, China).

2.9. Dual-luciferase reporter gene assay

The biological prediction website (http://www.targetscan.org) was employed to predict the target relationship between miR-494 and NQO1 and the binding site of miR-494 to NQO1 3′UTR, which was then identified using Dual luciferase reporter gene assay. The NQO1 3′UTR promoter region sequence containing the binding site of miR-494 was synthesized and the wild type (Wt) NQO1 3′UTR plasmid was constructed. Based on this plasmid, the mutant type (Mut) NQO1 3′UTR plasmid was constructed with mutation in binding site. The procedures were conducted according to the plasmid extraction kit (Promega Corporation, Madison, WI, USA). Luciferase reporter plasmids of Wt and Mut with correct sequences were separately co-transfected into HEK-293T cells (Beinuo Life Science, Shanghai, China) with mimic-NC or miR-494. After 48 h, the cells were collected and lysed, and the luciferase activity was determined with fluorometric assay kit (K800-200, Biovision, Mountain View, CA, USA). The luciferase activity was detected using Glomax 20/20 luminometer (Promega Corporation, Madison, WI, USA). The experiment was repeated for 3 times.

2.10. Statistical analysis

Statistical analysis was performed by the SPSS 21.0 software (IBM Corp., Armonk, NY, USA). Measurement data were expressed as mean ± standard deviation. The unpaired t-test was used for comparisons of data with normal distribution and homogeneity of variance between two groups, comparison of data with normal distribution but without homogeneity of variance among multiple groups were assessed using the Welch’s t-test. The intragroup comparison of data was analyzed by paired t-test. A value of < 0.05 was considered to be statistically significant.

Results

3.1. Upregulation of miR-494 increases ratio of right lung W/D in lung tissues of ARDS rats

The sepsis-associated ARDS rat models were established with cecal ligation and puncture (CLP). There was no death of rats in each group at the end of the experiment. Rats in the sham group showed normal activity, no shortness of breath, no heart beat faster and no inflammatory exudate from the five sense organs with normal water feeding. In comparison to rats in the sham group, rats in the ALI group showed less activity, shortness of breath, heart beat faster, slow response, bloody discharge from the mouth and eye, and less intake of water. After 24 h of model establishment, compared with the sham group, the ALI group showed an increased ratio of right lung W/D (p < 0.05) (Fig. 1A). Compared with the mimic-NC group, the miR-494 mimic group also showed an increased ratio of right lung W/D (p < 0.05) (Fig. 1B). And the miR-494 mimic + SFN group exhibited an increased ratio of right lung W/D than the miR-494 mimic group (p < 0.05) (Fig. 1C). These findings showed that upregulation of miR-494 increased ratio of right lung W/D, while the activation of Nrfl2 signaling pathway may reverse this contributory effect.

3.2. Upregulation of miR-494 exacerbates ALI in lung tissues of ARDS rats

HE staining was adopted to detect histological changes of left lung tissues in each group. The rats in the sham group exhibited clear and complete lung structure, clear alveolar structure, thinner alveolar wall, no inflammatory infiltration, exudate or bloody discharge from the alveolar cavity. The rats in the ALI group showed damaged alveolar structure with widen gap between alveoli, inflammatory cell infiltration and exudates and less bleeding in the interstitium of alveoli, and formation of transparent membrane within alveolar cavity. The rats in the miR-494 mimic group showed more severe and larger lesion than those in the mimic-NC group. The rats in the miR-494 mimic + SFN group showed clear and complete alveolar structure and less inflammatory cell infiltration in the interstitium of alveoli, which revealed improvement of ALI in comparison to the miR-494 mimic group (Fig. 2). These findings indicated that upregulation of miR-494 increased inflammatory cell infiltration and enhanced ALI, while the
activation of Nrf2 signaling pathway could attenuate ALI.

3.3. Upregulation of miR-494 decreases positive rate of NQO1 protein expression in lung tissues of ARDS rats

The positive rate of NQO1 protein expression was detected by immunohistochemistry assay to explore the effect of miR-494 on NQO1. In the sham group, NQO1 was mainly expressed in bronchial epithelial cells and alveolar epithelial cells in left lung tissues. After 24 h of model establishment, the positive rate of NQO1 protein expression in the ALI group was obviously lower than that in the sham group ($p < 0.05$) (Fig. 3A–B). The positive rate of NQO1 protein expression in the miR-494 mimic group was obviously lower than that in the mimic-NC group ($p < 0.05$) (Fig. 3C–D). The rats in miR-494 mimic + SFN group showed brown NQO1 expression particles in bronchial epithelial cells and alveolar epithelial cells in left lung tissues, and showed higher positive rate of NQO1 protein expression in comparison to miR-494 mimic group (Fig. 3E–F). These findings indicated that upregulation of miR-494 reduced positive rate of NQO1 protein expression which could be elevated by the activation of Nrf2 signaling pathway.

3.4. Upregulation of miR-494 promotes while activation of Nrf2 signaling pathway inhibits in inflammatory response in lung tissues of ARDS rats

The levels of IL-1β, IL-6, and TNF-α in the BALF were determined using ELISA to explore the effect of miR-494 and Nrf2 signaling pathway on inflammatory response. Compared with the sham group, the ALI group revealed higher levels of IL-1β, IL-6, and TNF-α (all $p < 0.05$). The levels of IL-1β, IL-6, and TNF-α in the miR-494 mimic group were significantly higher than those in the mimic-NC group (all $p < 0.05$). Compared with the miR-494 mimic group, the levels of IL-1β, IL-6, and TNF-α in the miR-494 mimic + SFN group showed obvious reductions (all $p < 0.05$) (Fig. 4). These findings indicated that upregulation of miR-494 induced higher inflammatory response, while activation of Nrf2 signaling pathway reversed this induction.

3.5. MiR-494 induces oxidative stress via NQO1-dependent inhibition of Nrf2 signaling pathway in lung tissues of ARDS rats

To investigate the effect of miR-494 on NQO1, Nrf2 signaling pathway and oxidative stress, RT-qPCR and Western blot analysis were adopted to detect miR-494 level, mRNA levels of NQO1, SOD and CAT, and protein levels of NQO1, HO-1 and Nrf2. Compared with the sham group, the ALI group exerted an increased miR-494 level but reduced of NQO1 protein and mRNA levels, as well as reduced Nrf2 protein level in W/D W/D W/D Sham ALI mimic-NC miR-494 mimic miR-494 mimic + SFN Fig. 1. Overexpressed miR-494 or inactivated Nrf2 signaling pathway increases ratio of right lung W/D in ARDS rats. Panel A, the ratio of right lung W/D increased in the ALI rats compared with rats in the sham group; *, $p < 0.05$, compared with the sham group; Panel B, the ratio of right lung W/D increased in the miR-494 mimic group than in the mimic-NC group; *, $p < 0.05$, compared with the mimic-NC group; Panel C, the ratio of right lung W/D decreased in the miR-494 mimic + SFN groups than in the miR-494 mimic group; *, $p < 0.05$, compared with the miR-494 mimic group; Nrf2, nuclear factor E2 p45-related factor 2; W/D, wet/dry; miR-494, microRNA-494; ALI, acute lung injury; SFN, sulforaphane. The measurement data were expressed as mean ± standard deviation, the comparison of which between two groups was analyzed using t-test; n = 8.

Fig. 2. HE staining reflects that upregulation of miR-494 enhances while activation of Nrf2 signaling pathway attenuates ALI in lung tissues of ARDS rats ($\times$ 200). The treatment of miR-494 mimic resulted in more severe and larger lesion in ALI rats. SFN was applied to activate Nrf2 signaling pathway, contributing to relieved ALI, with clear and complete alveolar structure and less inflammatory cell infiltration in the interstitium of alveoli. HE, hematoxylin-eosin; ALI, acute lung injury; NC, negative control; SFN, sulforaphane; Nrf2, nuclear factor E2 p45-related factor 2.
Fig. 3. Immunohistochemistry assay shows that miR-494 decreases while the activation of Nrf2 signaling pathway increases the positive rate of NQO1 protein expression in the left lung tissues of ARDS rats (×200). Panel A, under the microscope, NQO1 was mainly expressed in bronchial epithelial cells and alveolar epithelial cells in left lung tissues of rats in the sham group (×200); Panel B, positive rate of NQO1 protein expression was decreased in the ALI group; *, p < 0.05, compared with the sham group; Panel C, immunohistochemistry assay was applied for the observation of positive NQO1 protein expression in the mimic-NC and miR-494 mimic groups (×200); Panel D, positive rate of NQO1 protein expression was reduced in the miR-494 mimic group; *, p < 0.05, compared with the mimic-NC group; Panel E, under the microscope, rats in miR-494 mimic + SFN group showed brown NQO1 expression particles in bronchial epithelial cells and alveolar epithelial cells in left lung tissues (×200); Panel F, positive rate of NQO1 protein expression was increased in the miR-494 mimic + SFN group; *, p < 0.05, compared with the miR-494 mimic group; NC, negative control; miR-494, microRNA-494; NQO1, NAD(P)H:quinone oxidoreductase 1; ALI, acute lung injury; SFN, sulforaphane. The measurement data were expressed as mean ± standard deviation, the comparison of which between two groups was analyzed using t-test; n = 8; the experiment was repeated 3 times. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. ELISA shows that upregulation of miR-494 inhibits while activation of Nrf2 signaling pathway promotes inflammatory response. Panel A, the levels of IL-1β, IL-6, and TNF-α was increased in the ALI groups, which suggested an increased inflammatory response to ALI; *, p < 0.05, compared with the sham group; Panel B, the levels of IL-1β, IL-6, and TNF-α was increased in the miR-494 mimic group compared with the mimic-NC group; *, p < 0.05, compared with the mimic-NC group; Panel C, the levels of IL-1β, IL-6, and TNF-α was lower in the miR-494 mimic + SFN group than in the miR-494 mimic group; *, p < 0.05, compared with the miR-494 mimic group; NC, negative control; ALI, acute lung injury; miR-494, microRNA-494; ELISA, Enzyme-linked immunosorbent assay; IL, interleukin; TNF-α, tumor necrosis factor; SFN, sulforaphane; Nrf2, nuclear factor E2 p45-related factor 2. The measurement data were expressed as mean ± standard deviation, the comparison of which between two groups was analyzed using t-test; n = 8; the experiment was repeated 3 times.
3.6 MiR-494 negatively targets NQO1

The website http://www.targetscan.org predicted that NQO1 was the target gene of miR-494 (Fig. 6A), which was further verified by dual luciferase reporter gene assay. Compared with the mimic-NC, luciferase activity of Wt NQO1 3′-UTR was significantly inhibited by miR-495 mimic (p < 0.05), but no difference was found in Mut NQO1 3′-UTR (p > 0.05). Therefore, we reached a conclusion that miR-495 could bind to NQO1 3′-UTR and downregulate NQO1 expression (Fig. 6B).

4. Discussion

The development of ARDS results in a high mortality rate and significant physical and cognitive impairment [21]. In recent years, miRs have been reported to exert potential relevance for the diagnosis and therapy of ARDS [22]. In order to test the hypothesis that miR-494 may promote ALI by targeting Nrf2 signaling pathway in sepsis-associated ARDS rats, miR-494 mimic was introduced into rats to analyze the function of miR-494 on the ALI. Besides, rats were treated with sulforaphane, an activator of Nrf2 signaling pathway so as to show...
the regulation of miR-494 via the Nrf2 signaling pathway. At last, these results came to a conclusion that miR-494 negatively targeted NQO1, to inactivate the Nrf2 signaling pathway, thereby enhancing ALI in sepsis-associated ARDS rats.

Initially, the present study demonstrated that miR-494 was upregulated in rat models of sepsis-associated ARDS. It is reported that miRNA expression served as biomarkers for better disease diagnosis and risk estimate for ARDS [23]. Rao et al. have revealed that miR-28 expression is negatively correlated with Nrf2 expression. In line with these reports, this study also reached a conclusion that miR-494 negatively targeted NQO1, to inactivate the Nrf2 signaling pathway, thereby enhancing ALI in sepsis-associated ARDS rats.

Fig. 6. Dual-luciferase reporter gene assay shows that miR-494 binds to NQO1 3'-UTR. Panel A, The binding site of miR-494 in NQO1 3'-UTR was predicted on the website http://www.targetscan.org; Panel B, Measurement of luciferase activity was performed by dual-luciferase reporter gene assay to verify the target relationship between miR-494 and NQO1, indicating that miR-494 binds to NQO1 3'-UTR; *, p < 0.05, compared with the mimic-NC group; WT, wild type; MUT, mutant type; NC, negative control; miR-494, microRNA-494; NQO1, NAD(P)H:quinone oxidoreductase 1. The measurement data were expressed as mean ± standard deviation, the comparison of which between two groups was analyzed using t-test; the experiment was repeated three times.

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