European Review for Medical and Pharmacological Sciences 2020; 24: 9712-9720

MiR-181-5p protects mice from sepsis *via* repressing HMGB1 in an experimental model

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Abstract. – OBJECTIVE: Lentivirus-delivered microRNA (miR) has been reported to improve survival outcomes and organ dysfunction. The present study is aimed to explore whether sepsis-associated miR, miR-181-5p, could mitigate sepsis-induced inflammation and organ injury by the lentivirus-expressing system.

MATERIALS AND METHODS: Cecal ligation and puncture (CLP)-operated mice were treated with lentivirus-expressing miR-181-5p (miR-agomir) 7 days before surgical operation by intravenous injection. Acute renal and hepatic injuries were assessed using specific biomarkers. Survival outcomes were evaluated following CLP operation within 72 hours.

RESULTS: Lentivirus-delivered miR-181-5p improves survival outcomes of CLP-induced septic mice. The rescue of miR-181-5p expression by lentivirus expression vector protects against sepsis-induced renal and hepatic dysfunction. Sepsis-triggered inflammatory response and the release of HMGB1 level could be attenuated by miR-agomir administration. We also found that HMGB1 was a direct target of miR-181-5p, and that the overexpression of miR-81-5p led to a significant decrease in HMGB1 protein expression.

CONCLUSIONS: miR-181-5p-mediated protective effects in septic mice were modulated, at least partially, through post-transcriptional repression of HMGB1 protein expression. The findings suggest that miR-181-5p may function as an HMGB1 antagonist for alleviating sepsis-induced systemic inflammatory diseases.

Key Words:
MiR-181-5p, Sepsis, HMGB1, Acute kidney injury, Inflammation.

Abbreviations

miR = microRNA; CLP = cecal ligation and puncture; AKI = acute kidney injury; 3'-UTR = 3'-untranslated region; HMGB1 = high-mobility group box protein 1; TNF-α = tumor necrosis factor-α; IL-1β = interleukin-1β.

Introduction

Sepsis is a common and frequently systemic inflammatory response syndrome and has been consistently associated with acute injuries in multiple organs, including acute kidney injury (AKI)[1]. Sepsis is recognized as a foremost precipitant of AKI, which is accounting for approximately 50% septic patients[2]. At present, the molecular mechanisms underlying the sepsis-caused AKI are incompletely understood. Notably, microRNAs (miRs) are implicated in this pathological process[3-5]. MiR-21, miR-590-3p, and miR-124 confer renoprotection against sepsis-induced AKI[3-5]. In contrast to those miRs, miR-34a, miR-106a, and miR-142-3p aggravate sepsis-induced AKI[6-8]. Mechanistically, miRs as a class of small non-coding RNAs perform their function as post-transcriptional regulators *via* binding to the 3'-untranslated region (3'-UTR) of the target genes to repress protein translation[9], which is frequently reported in the pathogenesis of AKI[10,11].

High-mobility group box protein 1 (HMGB1), as a DNA chaperone, usually resides in the nucleus and participates in regulating chromosomal structure and DNA biology[12]. HMGB1, as a damage-associated mediator, is released from necrotic cells and mediates sepsis-induced inflammatory response[13]. Acetylation modification of HMGB1 accelerates the development of sepsis-associated AKI *via* facilitating its nucleus-to-cytoplasm translocation and extracellular secretion in kidney cells[13]. Elevation of HMGB1 potentiates the release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6, in septic rat[14]. Deterioration of sepsis can exacerbate HMGB1 oxidation, which enhances the pro-inflammatory signaling path-

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way[15]. The findings suggest that HMGB1 plays a crucial role in sepsis-associated organ damage.

In the present study, our results showed that miR-181-5p was significantly declined in the kidneys of septic mice compared with those in the sham group. Next, miR-181-5p agomir was used to administrate cecal ligation and puncture (CLP)-operated mice to determine whether miR-181-5p protected against sepsis-induced AKI. Finally, we investigated whether HMGB1 as a direct target of miR-181-5p was involved in the pathogenesis of sepsis-associated AKI.

Material and Methods

Animal Experiments

Eight-week-old male C57BL/6J mice (body weight, 20 ± 2 g) were obtained from the Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All mice acclimated to the environment for 1 week before administration experiments and were fed in a temperature-controlled environment (temperature: 25 ± 2˚C; humidity: 60±5%) with an artificial 12-h light/dark cycle and were provided with free access to food and tap water. Septic mice were established using CLP operation described previously[16]. Mice were randomly divided into 4 groups as follows: sham group, CLP group, CLP-operated mice combined with miR-Con administration *via* tail-vein injection (CLP+miR-Con group); CLP-operated mice combined with miR-181-5p agomir treatment via tail-vein injection (CLP+miR-agomir group; n = 12 in each group). MiR-Con or miR-181-5p agomir administration was performed before 7 days of CLP operation (twice within 7 days). MiR-Con or miR-181-5p (1×10^8 plaque-forming units/100 μl) were delivered *via* lentivirus expression system and injected into mice by tail vein. The experiment was approved by the Ethics Committee of the Lanzhou University and the Emergency Medical Rescue Center of Gansu Province of China. In addition, survival outcomes of septic mice with miR-Con or miR-181-5p treatment within 72 hours were observed in another experiment (n = 12 in each group).

Sepsis-Related miRs Filtration

Sepsis-related miRs were filtrated using SurePrint G3 8 × 60K miR microarrays (Agilent, Santa Clara, CA, USA). Total RNA was extracted using miRNeasy Mini Kit (Qiagen, Dusseldorf, Nordrhein-Westfalen, Germany), according to the manufacturer's protocol. miRs were labeled with Hy3 or Hy5 fluorescence using the miRCURY™ Array Power Labeling Kit (Qiagen, Dusseldorf, Nordrhein-Westfalen, Germany) to obtain the fluorescent probe that can be hybridized with the chip. The labeled probe was hybridized with the miRCURY™ chip under the standard condition using the MAUI hybridization system. The fluorescence intensity of the chip was scanned with the Agilent chip scanner and analyzed using Agilent feature extraction software (version 12; Agilent, Santa Clara, CA, USA). The candidate miRs were screened based on the fold change ≥ 2 or ≤ -2, $p < 0.05$ and FDR < 0.05. Hierarchical clustering was used to determine similarity using complete linkage and euclidean distance using MeV software (version 4.2.6; Institute for Genomic Research, Rockville, MD, USA).

Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted using miRNeasy Mini Kit (Qiagen, Dusseldorf, Nordrhein-Westfalen, Germany), according to the manufacturer's protocol. TaqMan® RT kit and TaqMan® MicroRNA assay (Applied Biosystems, Foster City, CA, USA) were used to detect miRs expression levels using Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA). miRs expression levels were calculated using $2^{-\Delta\Delta Ct}$ method, as described previously[17], and U6 was used as an internal control. The primers for PCR were listed as follows: miR-181-5p forward 5'-GCGCAACATTCAACGCTGTCG-3' and reverse 5'-GTGCAGGGTCCGAGGT-3'; U6 forward 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'.

Enzyme-Linked Immunosorbent Assay (ELISA)

Blood urea nitrogen (BUN), serum creatinine (Cr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), HMGB1, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin 6 (IL-6) were measured using commercial kits from (Elabscience Biotechnology Co., Ltd, Wuhan, HuBei, China) with a SpectraMax M5 ELISA plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA), according to the manufacturer's instructions.

Western Blotting

Total protein was extracted using RIPA Lysis Buffer (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China). The concentration was determined using the Bicinchoninic Acid Kit for Protein Determination (Sigma-Aldrich, St. Louis, MO, USA). 50 μg total protein were separated by 10% SDS-PAGE gel and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The primary antibody for HMGB1 (cat. No: ab77302; dilution: 1: 1,000) was purchased from Abcam (Cambridge, UK). Horseradish peroxidase-conjugated secondary antibody was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Protein bands were visualized using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Signals were analyzed with Quantity One® software version 4.5 (Bio Rad Laboratories, Inc., Hercules, CA, USA). Anti-β-actin (cat. no. sc-130065; dilution: 1: 2,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was used to as the control antibody.

Cell Culture

Mouse glomerular mesangial cells (GMCs) and NCTC-1469 cells were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's Modified Eagle's Medium-F12 (DMEM-F12; Gibco, Grand Island, NY, USA) medium with 5 % fetal bovine serum (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified incubator (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 5% CO_2, 95% air atmosphere.

Luciferase Reporter Assay

HMGB1 with wild-type (WT) and mutant-type (MUT) 3'-UTR were synthesized by Sangon (Shanghai, China) and inserted into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA). For the Luciferase assay, GMCs and NCTC-1469 cells (1×10^5) were seeded into 24-wells and co-transfected with Luciferase reporter vectors containing WT or MUT 3'-UTR (0.5 μg) of HMGB1 combined with miR-Con or miR-181-5p mimics using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C for 48 h. Luciferase activity was measured using a Dual-Luciferase reporter assay kit (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China), according to the manufacturer's protocol. MiR-Con or miR-181-5p mimics were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, Guangdong, China).

Statistical Analysis

Data were presented as mean ± SD. Statistical analysis was performed using GraphPad Prism Version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Student *t*-test was used to analyze two-group differences. Inter-group differences were analyzed by one-way analysis of variance, followed by Tukey's post-hoc analysis. $p < 0.05$ was considered to indicate a statistically significant difference.

Results

MiR-181-5p is Decreased in the Kidneys of Septic Mice

To explore the role of miRs underlying sepsis-associated organ damage, differentially expressed miRs were evaluated using high-throughput miRs microarray analysis. Our results revealed that 198 abnormally expressed miRs were observed in the kidneys of septic mice compared with those sham-operated mice. Among the miRs, 94 were significantly downregulated, and 104 significantly upregulated in the kidneys of septic mice compared with those of sham-operated mice (Figure 1A). Interestingly, miR-181-5p (log2 fold change = -5.37) was the most sensitive miR in response to sepsis and focused in further studies. To validate miR-181-5p expression level by high-throughput analysis, we examined its expression in the kidneys using qRT-PCR assays. Consistent with the high-throughput result, RT-PCR corroborated that miR-181-5p was dramatically reduced in the kidneys of septic mice compared with those of sham-operated mice (Figure 1B).

Transfection with miR-181-5p Agomir Improves Survival of Septic Mice

The previous study shows that miR-125b protects mice against lethal sepsis[18]. To investigate the protective effect of miR-181-5p in septic mice, we established a miR-181-5p overexpressed vector delivering lentivirus *via* intravenous injection. First, sepsis-induced decrease in miR-181-5p expression level in the kidneys of septic mice was significantly elevated by the transfection of miR-

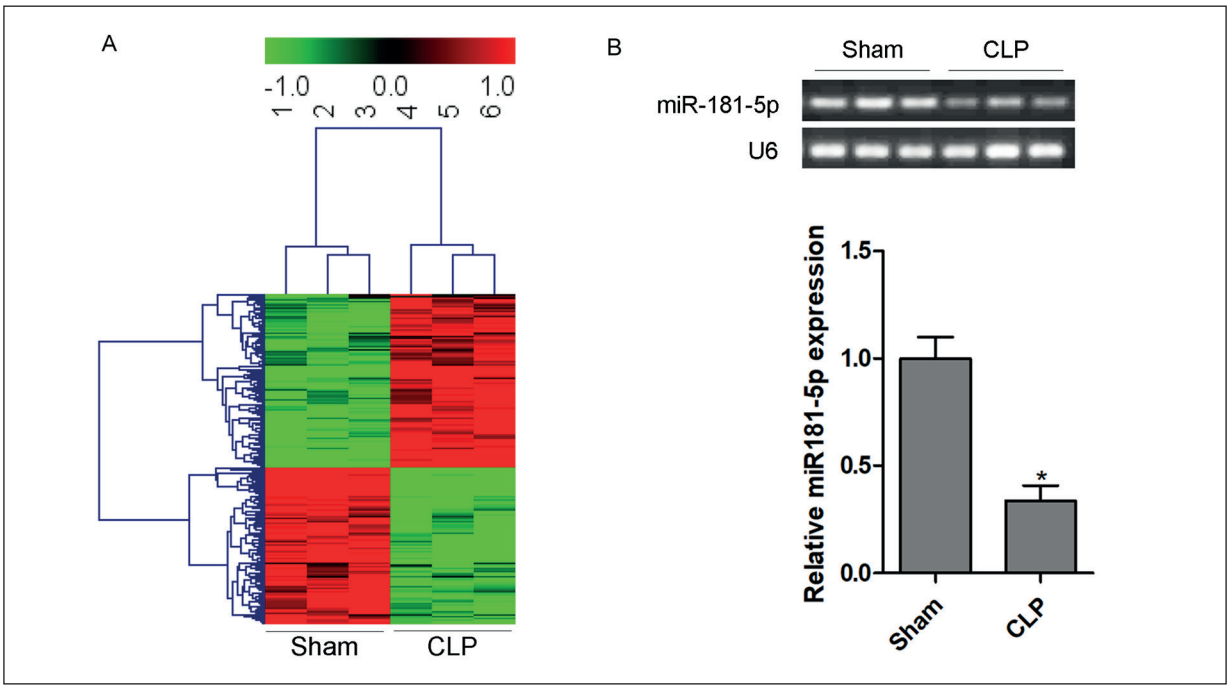

Figure 1. MiR-181-5p is decreased in the kidneys of septic mice. Differentially expressed miRs in the kidneys from sham-operated or CLP-operated mice were evaluated using miRs microarray analysis (**A**). qRT-PCR corroborated the expression of miR-181-5p in the kidney of septic mice and sham-operated mice (**B**). *$p < 0.05$.

181-5p agomir (Figure 2A). As shown in Figure 2B, CLP-operated led to 100% mortality in septic mice within 72 hours. In addition, the mortality in the CLP+miR-Con group was 91.7%. Compared with the CLP+miR-Con group, miR-181-5p transfection significantly improved the survival outcome of septic mice from 8.3% to 58.3%.

Transfection with MiR-181-5p Attenuates Renal and Hepatic Dysfunction

Sepsis as a serious syndrome, primarily causes acute renal and hepatic dysfunction[19]. Biomarkers of kidney injury, including BUN and serum Cr, were significantly increased in septic mice compared with those in sham-operated mice. Simultaneously, hepatic dysfunction was perceived in septic mice, reflecting that serum ALT and AST were increased in CLP-operated mice (Figure 2C and 2D). However, transfection of miR-181-5p agomir prevented sepsis-stimulated production of BUN, serum Cr, ALT and AST (Figure 2C and 2D).

Transfection with MiR-181-5p Agomir Represses the Release of HMGB1 and Proinflammatory Cytokine in Septic Mice

The inflammatory response is primarily initiated by sepsis to induce organ dysfunction[20]. In the process, proinflammatory cytokines, including TNF-α, IL-1β and IL-6, are urgently released into the peripheral circulatory system[20]. The previous study has also reported that HMGB1 contributes to systemic inflammation and multiple organ failure[21]. Our results indicated that serum HMGB1 levels were significantly increased after CLP operation in a time-dependent manner (Figure 3A). Unlike the change of serum HMGB1 levels, proinflammatory cytokines, TNF-α, IL-1β and IL-6, were significantly increased after CLP operation, but peaking at 12 h (Figure 3B, 3C and 3D). Collectively, transfection with miR-181-5p agomir into septic mice could decline the production of HMGB1, TNF-α, IL-1β and IL-6 in the serum (Figure 3A, 3B, 3C and 3D).

Transfection with MiR-181-5p Agomir Represses the Protein Expression of HMGB1 in the Kidneys and Livers of Septic Mice

HMGB1 as a proinflammatory stimulator has been continually reported in damaged organs[22,23]. The present study revealed that the protein expression of HMGB1 was significantly increased in both kidneys and livers of septic mice compared with those of sham-operated

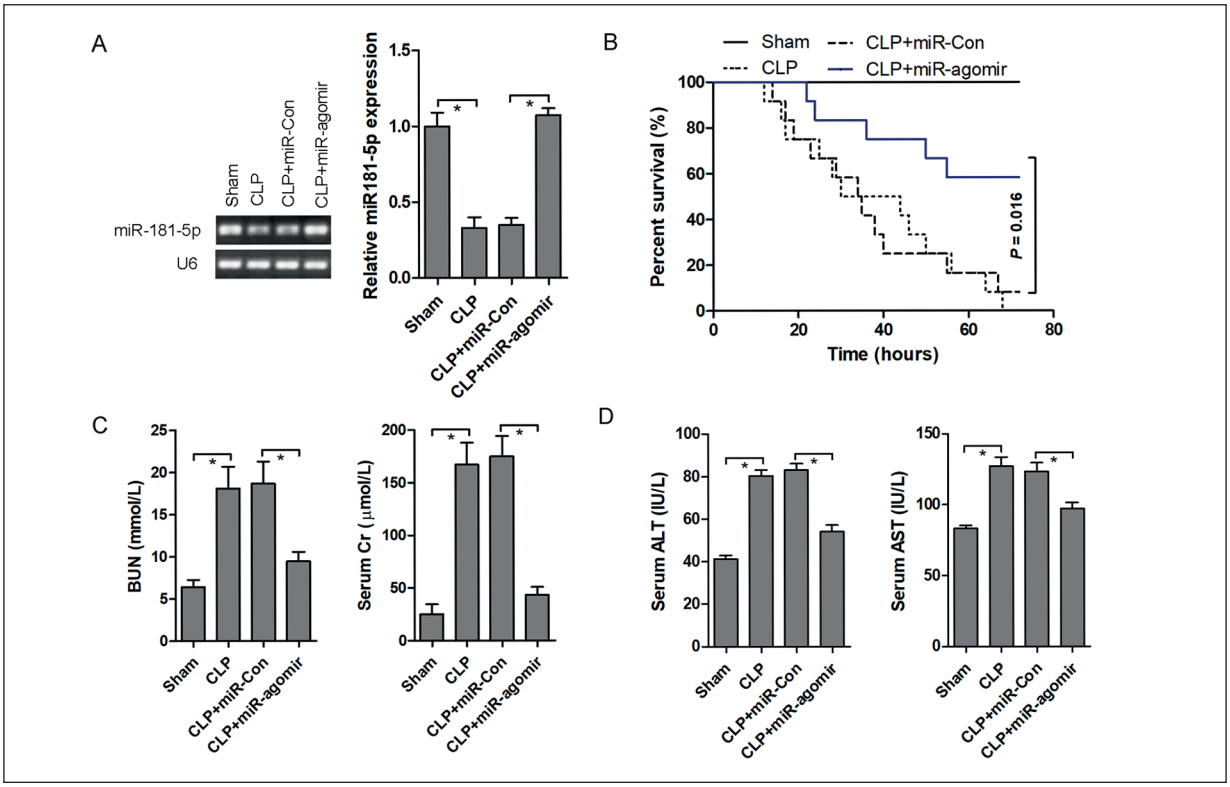

Figure 2. Transfection with miR-181-5p agomir improves survival and organ dysfunction of septic mice. After transfection with miR-Con or miR-181-5p agomir into septic mice, the expression levels of miR-181-5p were monitored using RT-qPCR (**A**); survival outcome of septic mice was evaluated using the Kaplan-Meier method with the log-rank test (**B**); acute renal (**C**) and hepatic (**D**) injuries biomarkers were assessed using ELISA assays. *$p < 0.05$.

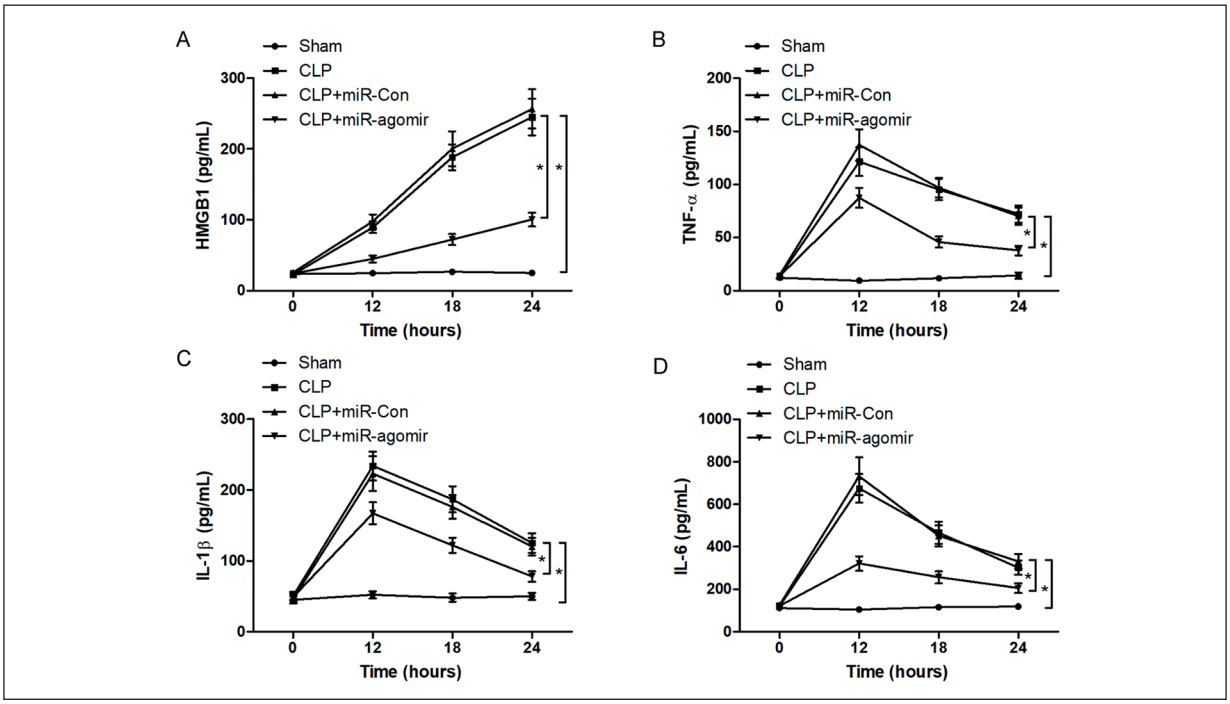

Figure 3. Transfection with miR-181-5p agomir represses the release of HMGB1 and proinflammatory cytokine in septic mice. After transfection with miR-Con or miR-181-5p agomir into septic mice, the serum levels of HMGB1 (**A**), TNF-α (**B**), IL-1β (**C**) and IL-6 (**D**) were assessed using ELISA assays. *$p < 0.05$.

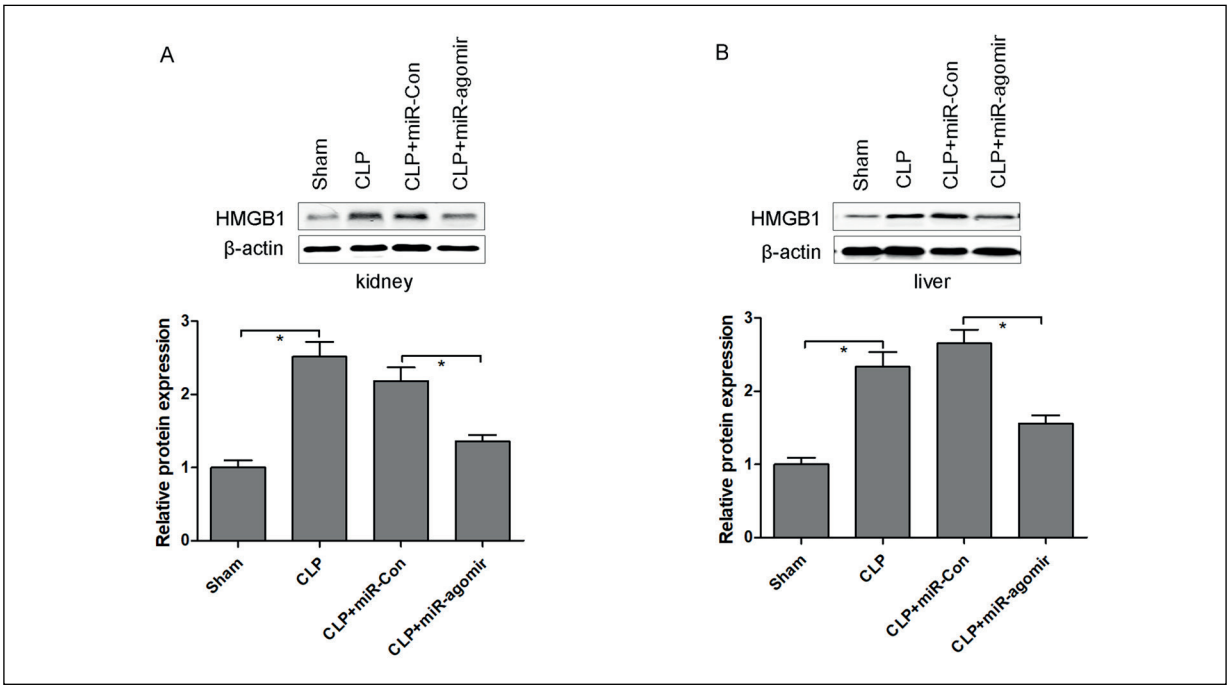

Figure 4. Transfection with miR-181-5p agomir represses the protein expression of HMGB1 in the kidney and liver of septic mice. After transfection with miR-Con or miR-181-5p agomir into septic mice, the protein expression of HMGB1 in kidney (**A**) and liver (**B**) was detected by Western blotting assays. *$p < 0.05$.

mice (Figure 4A and 4B). Intriguingly, it was showed that miR-181-5p agomir neutralized the sepsis-induced up-regulation of HMGB1 protein expression in both kidneys and livers (Figure 4A and 4B).

HMGB1 is a Direct Target of MiR-181-5p

Based on the above findings, we found that miR-181-5p could regulate HMGB1 expression in sepsis-induced inflammation and organ dysfunction. However, whether HMGB1 acts as a direct target of miR-181-5p is unclear. Bioinformatics algorithms showed that a conserved sequence in the 3'-UTR could bind with miR-181-5p, and the schematic diagram was presented as shown in Figure 5A. In addition, the Luciferase reporter assay was performed in mouse renal GMCs and normal mouse liver NCTC-1469 cells. After transfection with miR-181-3p mimics, the Luciferase activity was significantly reduced in GMCs and NCTC-1469 cells containing WT 3'-UTR of HMGB1 compared with those cells transfected with miR-Com. However, transfection with miR-181-3p mimics brought no evident change of Luciferase activities in GMCs and NCTC-1469 cells containing Mut 3'-UTR of HMGB1 (Figure 5B). Compared with those in the miR-Con group, transfection with miR-181-3p mimics into GMCs and NCTC-1469 cells significantly repressed the protein expression of HMGB1 (Figure 5C and 5D). The findings suggest that HMGB1 as a direct target of miR-181-5p could be regulated by miR-181-5p *via* post-transcriptional silencing.

Discussion

HMGB1 is a ubiquitous nuclear protein and performs multiple functions in the physiological and pathological processes, including DNA chaperone, the proinflammatory activity, hypertension, cardiac hypertrophy and apoptosis[21,24,25]. HMGB1 is acquainted as a proinflammatory mediator in organ or cell damage[26]. The over-expression of extracellular HMGB1 can stimulate the activation of resident immune cells in the adipose tissue to secrete additional HMGB1, which can maintain a chronic inflammatory state in adipose tissue[26,27]. HMGB1 translocation from the nucleus to the cytoplasm leads to inflammation-associated lung damage in an experimental mouse model[27]. HMGB1 is also testified as an early mediator of renal ischemia reperfusion and associated with renal inflammation and apoptotic cell death[28].

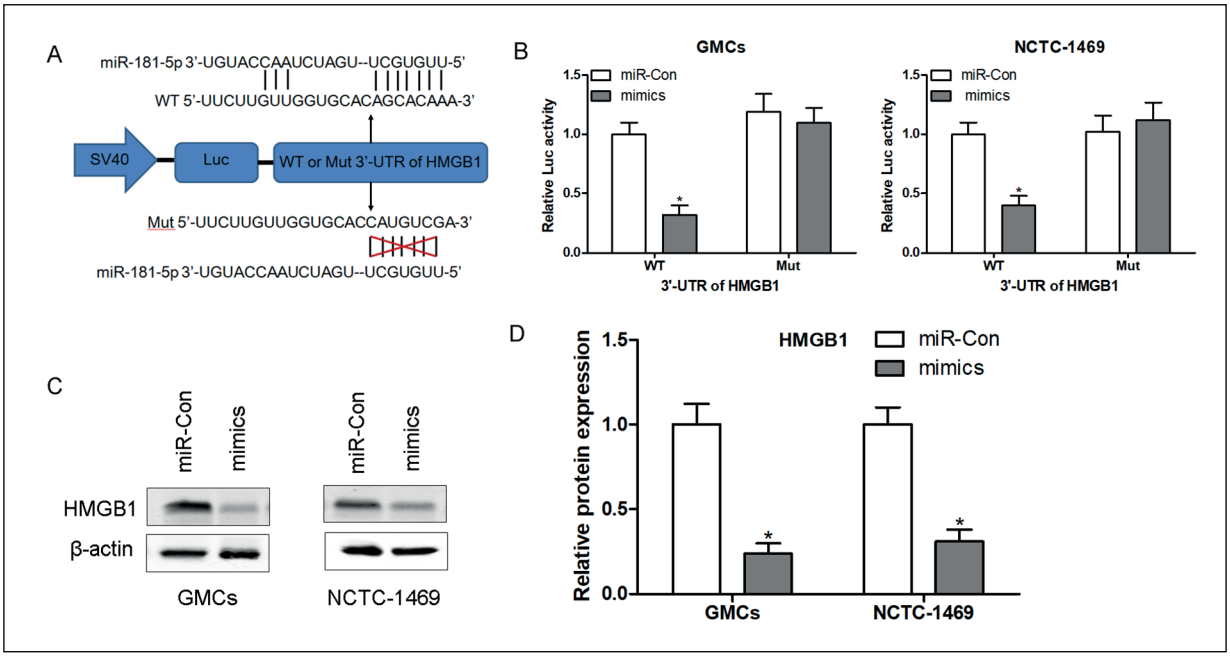

Figure 5. HMGB1 is a direct target of miR-181-5p. A conserved sequence in the 3'-UTR could be binded with miR-181-5p, which was predicted using bioinformatics algorithms (**A**). Luciferase reporter assay was performed in mouse renal GMCs and mouse normal liver NCTC-1469 cells (**B**). After transfection with miR-Con or miR-181-3p mimics into mouse renal GMCs and mouse normal liver NCTC-1469 cells, the protein expression of HMGB1 was detected by western blotting assays (**C** and **D**). *$p < 0.05$.

The suppression of HMGB1 represents a promising approach for the treatment of inflammation and organ damage in response to various stimuli[29-32]. So, HMGB1 antagonist, glycyrrhizin, helps overcome the brain, hepatic and neural injury *via* inhibiting extracellular HMGB1 cytokine activity[30-32]. The findings suggest that HMGB1 is a meritorious therapeutic target for protecting against multiple organ dysfunction.

HMGB1, as a late mediator of lethal systemic inflammation, is implicated in sepsis-associated pathological changes of tissues[12,33]. Plasma or serum HMGB1 level is increased in critically ill patients with septic shock or CLP-induced sepsis mice[13,34,35]. Our results, consistent with the previous reports, validated that HMGB1 was continuously released into peripheral blood of septic mice in a time-dependent manner. We also observed that the protein expression of HMGB1 was significantly elevated in the kidney and liver of septic mice compared to those in the sham-operated mice. Interestingly, our results exhibited that sepsis specific miR-181-5p might be a promising gene therapy strategy for preventing HMGB1-triggered inflammatory response and organ dysfunction in septic mice.

The previous study suggests that miR-181-5p declines in the peripheral blood and articular cartilage of osteoarthritis (OA) patients and functions as a marker for OA screening[36], indicating that the downregulation of miR-181-5p may exacerbate the progression of OA. In addition, exosomes-derived miR-181-5p possesses an anti-fibrotic activity in liver *via* the enhancement of autophagy[37]. Considering the multiple roles of miR-181-5p in organ damage, the role of miR-181-5p was investigated in septic mice. Our results demonstrated that lentivirus-transmissive miR-181-5p was employed mainly to improve inflammation and acute organ dysfunction, and HMGB1, as a trigger for inflammation, had been wiped out by overexpression of miR-181-5p in septic mice. Mechanistically, we found that HMGB1 was a direct target of miR-181-5p and therefore could be post-transcriptionally repressed by overexpression of miR-181-5p in septic mice.

Conclusions

Our study presents that lentivirus-delivered miR-181-5p improves survival outcomes of CLP-induced septic mice. Moreover, the rescue of miR-181-5p expression by lentivirus expression

vector protects against sepsis-induced renal and hepatic dysfunction. Sepsis-triggered inflammatory response and the release of HMGB1 level could be attenuated by overexpression of miR-181-5p. Furthermore, the underlying molecular mechanism of miR-181-5p-mediated protective effects in septic mice was modulated, at least partially, through post-transcriptional repression of HMGB1 protein expression. Therefore, miR-181-5p may function as an HMGB1 antagonist for alleviating sepsis-induced systemic inflammatory diseases.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

The study was approved by the Ethics Committee of the Lanzhou University and the Emergency Medical Rescue Center of Gansu Province of China.

Authors' Contribution

Study design was performed by X-M, J-Q and X-G; literature research, data acquisition and data analysis were performed by X-M, J-Q and X-G; manuscript preparation and manuscript editing were performed by XM, JQ and XG; manuscript was revised by XM, JQ and XG; cell and animal experiments were performed by X-M, J-Q and X-G; final approval of the version to be published by XM, JQ and XG.

References

1) Martensson J, Bellomo R. Sepsis-induced acute kidney injury. Crit Care Clin 2015; 31: 649-660.
2) Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Ronco C. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA 2005; 294: 813-818.
3) Pan T, Jia P, Chen N, Fang Y, Liang Y, Guo M, Ding X. delayed remote ischemic preconditioning confers renoprotection against septic acute kidney injury via exosomal miR-21. Theranostics 2019; 9: 405-423.
4) Ma J, Li YT, Zhang SX, Fu SZ, Ye XZ. MiR-590-3p Attenuates acute kidney injury by inhibiting tumor necrosis factor receptor-associated factor 6 in septic mice. Inflammation 2019; 42: 637-649.
5) Li XY, Zhang YQ, Xu G, Li SH, Li H. miR-124/MCP-1 signaling pathway modulates the protective effect of itraconazole on acute kidney injury in a mouse model of disseminated candidiasis. Int J Mol Med 2018; 41: 3468-3476.
6) Shen Y, Yu J. MiR-106a aggravates sepsis-induced acute kidney injury by targeting THBS2 in mice model. Acta Cir Bras 2019; 34: e201900602.
7) Liu X, Hong C. Downregulation of lncRNA TUG1 contributes to the development of sepsis-associated acute kidney injury via regulating miR-142-3p/sirtuin 1 axis and modulating NF-kappaB pathway. J Cell Biochem 2019; 120: 11331-11341.
8) Jiang ZJ, Zhang MY, Fan ZW, Sun WL, Tang Y. Influence of lncRNA HOTAIR on acute kidney injury in sepsis rats through regulating miR-34a/Bcl-2 pathway. Eur Rev Med Pharmacol Sci 2019; 23: 3512-3519.
9) Ueda T, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM. Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. Lancet Oncol 2010; 11: 136-146.
10) Wilflingseder J, Jelencsics K, Bergmeister H, Sunzenauer J, Regele H, Eskandary F, Reindl-Schwaighofer R, Kainz A, Oberbauer R. miR-182-5p inhibition ameliorates ischemic acute kidney injury. Am J Pathol 2017; 187: 70-79.
11) Chun N, Coca SG, He JC. A protective role for microRNA-688 in acute kidney injury. J Clin Invest 2018; 128: 5216-5218.
12) Yang L, Xie M, Yang M, Yu Y, Zhu S, Hou W, Kang R, Lotze MT, Billiar TR, Wang H, Cao L, Tang D. PKM2 regulates the Warburg effect and promotes HMGB1 release in sepsis. Nat Commun 2014; 5: 4436.
13) Wei S, Gao Y, Dai X, Fu W, Cai S, Fang H, Zeng Z, Chen Z. SIRT1-mediated HMGB1 deacetylation suppresses sepsis-associated acute kidney injury. Am J Physiol Renal Physiol 2019; 316: F20-f31.
14) Zhao F, Fang Y, Deng S, Li X, Zhou Y, Gong Y, Zhu H, Wang W. Glycyrrhizin protects rats from sepsis by blocking HMGB1 signaling. Biomed Res Int 2017; 2017: 9719647.
15) Abdulmahdi W, Patel D, Rabadi MM, Azar T, Jules E, Lipphardt M, Hashemiyoon R, Ratliff BB. HMGB1 redox during sepsis. Redox Biol 2017; 13: 600-607.
16) Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009; 4: 31-36.
17) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
18) Ma H, Wang X, Ha T, Gao M, Liu L, Wang R, Yu K, Kalbfleisch JH, Kao RL, Williams DL, Li C. MicroRNA-125b prevents cardiac dysfunction in polymicrobial sepsis by targeting TRAF6-mediated nuclear factor kappaB activation and p53-mediated apoptotic signaling. J Infect Dis 2016; 214: 1773-1783.
19) Garofalo AM, Lorente-Ros M, Goncalvez G, Carriedo D, Ballen-Barragan A, Villar-Fernandez A, Penuelas O, Herrero R, Granados-Carreno R, Lorente JA. Histopathological changes of organ

dysfunction in sepsis. Intensive Care Med Exp 2019; 7: 45.

20) Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol 2013; 13: 862-874.

21) Yang H, Antoine DJ, Andersson U, Tracey KJ. The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. J Leukoc Biol 2013; 93: 865-873.

22) Erdal M, Altunkaynak BZ, Kocaman A. The role of HMGB1 in liver inflammation in obese rats. Biotech Histochem 2019; 94: 449-458.

23) Zhang W, Wang Y, Kong Y. Exosomes derived from mesenchymal stem cells modulate miR-126 to ameliorate hyperglycemia-induced retinal inflammation via targeting HMGB1. Invest Ophthalmol Vis Sci 2019; 60: 294-303.

24) Zhang L, Liu M, Jiang H, Yu Y, Yu P, Tong R, Wu J, Zhang S, Yao K, Zou Y, Ge J. Extracellular high-mobility group box 1 mediates pressure overload-induced cardiac hypertrophy and heart failure. J Cell Mol Med 2016; 20: 459-470.

25) Tang Z, Jiang M, Ou-Yang Z, Wu H, Dong S, Hei M. High mobility group box 1 protein (HMGB1) as biomarker in hypoxia-induced persistent pulmonary hypertension of the newborn: a clinical and in vivo pilot study. Int J Med Sci 2019; 16: 1123-1131.

26) Zhang J, Zhang L, Zhang S, Yu Q, Xiong F, Huang K, Wang CY, Yang P. HMGB1, an innate alarmin, plays a critical role in chronic inflammation of adipose tissue in obesity. Mol Cell Endocrinol 2017; 454: 103-111.

27) Liu Q, Xie W, Wang Y, Chen S, Han J, Wang L, Gui P, Wu Q. JAK2/STAT1-mediated HMGB1 translocation increases inflammation and cell death in a ventilator-induced lung injury model. Lab Invest 2019;

28) Seo MS, Kim HJ. Ethyl pyruvate directly attenuates active secretion of HMGB1 in proximal tubular cells via Induction of heme oxygenase-1. J Clin Med 2019; 8: 629.

29) Musumeci D, Roviello GN, Montesarchio D. An overview on HMGB1 inhibitors as potential therapeutic agents in HMGB1-related pathologies. Pharmacol Ther 2014; 141: 347-357.

30) Li YJ, Wang L, Zhang B, Gao F, Yang CM. Glycyrrhizin, an HMGB1 inhibitor, exhibits neuroprotective effects in rats after lithium-pilocarpine-induced status epilepticus. J Pharm Pharmacol 2019; 71: 390-399.

31) Ieong C, Sun H, Wang Q, Ma J. Glycyrrhizin suppresses the expressions of HMGB1 and ameliorates inflammative effect after acute subarachnoid hemorrhage in rat model. J Clin Neurosci 2018; 47: 278-284.

32) Hua S, Ma M, Fei X, Zhang Y, Gong F, Fang M. Glycyrrhizin attenuates hepatic ischemia-reperfusion injury by suppressing HMGB1-dependent GSDMD-mediated kupffer cells pyroptosis. Int Immunopharmacol 2019; 68: 145-155.

33) Deng M, Scott MJ. Location is the key to function: HMGB1 in sepsis and trauma-induced inflammation. J Leukoc Biol 2019; 106: 161-169.

34) Gibot S, Massin F, Cravoisy A, Barraud D, Nace L, Levy B, Bollaert PE. High-mobility group box 1 protein plasma concentrations during septic shock. Intensive Care Med 2007; 33: 1347-1353.

35) Gil M, Kim YK, Hong SB, Lee KJ. Naringin decreases TNF-alpha and HMGB1 release from LPS-stimulated macrophages and improves survival in a CLP-induced sepsis mice. PLoS One 2016; 11: e0164186.

36) Xia S, Tian H, Fan L, Zheng J. Peripheral blood miR-181-5p serves as a marker for screening patients with osteoarthritis by targeting TNFalpha. Clin Lab 2017; 63: 1819-1825.

37) Qu Y, Zhang Q, Cai X, Li F, Ma Z, Xu M, Lu L. Exosomes derived from miR-181-5p-modified adipose-derived mesenchymal stem cells prevent liver fibrosis via autophagy activation. J Cell Mol Med 2017; 21: 2491-2502.