**MiR-425-5p protects against acute kidney injury in** **septic aging mice through targeting toll-like receptor 4**

**Running title:** Role of miR-425-5p in AKI.

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**Abstract**

**Objective** Acute kidney injury (AKI) is one of the complications of sepsis with the highest mortality. MiR-425-5p was involved in the regulation of septic liver damage, but its role in AKI was unknown. This study aimed to investigate the effect of miR-425-5p in septic AKI and underlying mechanism.

**Methods** This study established the septic aging mouse model by cecal ligation and puncture (CLP) method, and treated human proximal tubular epithelial (HK-2) cells by lipopolysaccharide (LPS) to induce an in vitro model of AKI. The dataset GSE94717 was downloaded from GEO database. Differentially expressed miRNAs were screened and identified. The miR-425-5p expression was detected in mice and cells. The interaction between miR-425-5p and toll-like receptor 4 (TLR4) was explored. Then, the effects of miR-425-5p inhibition or overexpression on TLR4 level, cell viability and production of cytokines in LPS-stimulated HK-2 cells and model mice were tested.

**Results** The results showed that levels of renal injury markers such as blood urea nitrogen (BUN) and serum creatinine (Scr), as well as production of pro-inflammatory cytokines were significantly elevated in septic mice, while miR-425-5p expression showed adverse results. Overexpression of miR-425-5p enhanced cell viability, while reduced TLR4 and pro-inflammatory cytokine levels, whereas these effects were reversed by TLR4 overexpression. Moreover, TLR4 was a target of miR-425-5p, and overexpression of miR-425-5p in vivo reduced TLR4 level and alleviated AKI.

**Conclusion** We concluded thatmiR-425-5p alleviated septic AKI via targeting TLR4, indicative a potential therapy target for AKI treatment.

**Keywords：** Sepsis, acute kidney injury, cecal ligation and puncture (CLP), miR-425-5p, TLR4

**Introduction**

Sepsis is considered as a dysregulated systemic response to infection with high morbidity and mortality worldwide [[1](#_ENREF_1" \o "van der Poll, 2017 #161), [2](#_ENREF_2" \o "Mayr, 2014 #162)]. Acute kidney injury (AKI) is a fatal complication of sepsis, which accounts for about 50% of cases [[3](#_ENREF_3" \o "Poston, 2019 #163)]. According to a previous study, the sepsis-induced AKI significantly increased the mortality of septic patients [[4](#_ENREF_4" \o "Gomez, 2016 #164)]. No effective drugs to prevent human sepsis-induced AKI are available at present [[5](#_ENREF_5" \o "Umbro, 2016 #165), [6](#_ENREF_6" \o "Sun, 2019 #7)]. The pathways of septic AKI include inflammation and changes in renal tubular epithelial cells to injury [[7](#_ENREF_7" \o "Zarbock, 2014 #8)]. However, the mechanism is complex and has not been fully illustrated. Moreover, people over 65 years old account for 60 percent of sepsis patients with a shorter servival rate and organ dysfunction [[8](#_ENREF_8" \o "Martin, 2003 #43), [9](#_ENREF_9" \o "Inoue, 2013 #44)]. Therefore, it is of great significance to explore ageing-related septic AKI.

MicroRNAs (miRNAs) are small endogenous non-coding RNAs that inhibit gene expression by binding to the 3′-untranslated region (3′-UTR) of target genes, which have been identified to be involved in regulation of sepsis [[10-12](#_ENREF_10" \o "Ge, 2018 #1)]. Numerous studies have claimed that aberrant miRNA expressions are closely related to inflammatory response in sepsis [[13-15](#_ENREF_13" \o "Yu, 2019 #169)]. For instance, miR-214 restrained the production of inflammatory cytokines in septic mice, as well as alleviated the myocardial injury [[14](#_ENREF_14" \o "Ge, 2018 #170)]. And that, miR-25 inhibits sepsis-induced cardiomyocyte apoptosis by suppressing the inflammatory response [[15](#_ENREF_15" \o "Yao, 2018 #173)]. MiR-425-5p, a member of miRNAs family, has been shown to regulate the development of various cancers, including breast cancer [[16](#_ENREF_16" \o "Xiao, 2019 #174)], gastric cancer [[17](#_ENREF_17" \o "Yan, 2017 #175)], prostate cancer [[18](#_ENREF_18" \o "Liu, 2019 #176)],et al. Furthermore, it has been demonstrated that miR-425-5p is down-regulated in LPS-induced septic mice, and overexpression of miR-425-5p inhibits the RIP1-mediated necroptotic signaling cascades and inflammation, and eventually alleviated the liver injury [[19](#_ENREF_19" \o "Gu, 2020 #177)]. However, the role of miR-425-5p in sepsis-induced AKI remains unclear.

Toll-like receptor 4 (TLR4) activates the innate immune system in response to exogenous microbial products, which may lead to collateral damage of host tissue and organ dysfunction [[20](#_ENREF_20" \o "Anderberg, 2017 #178)]. A number of studies have confirmed that the expression of TLR4 in sepsis is alterant and regulated by miRNAs. For example, TLR4 was up-regulated in macrophages under LPS treatment, and its expression was negatively regulated by miR-326 [[21](#_ENREF_21" \o "Wang, 2020 #194)]; TLR4 level was increased in lung tissues of septic mice, while miR-139-5p overexpression inhibited TLR4 level and alleviated lung injury of sepsis-induced mice [[22](#_ENREF_22" \o "Zhang, 2021 #180)]. However, whether miR-425-5p could regulate septic AKI through TLR4 is unknown.

Here, we investigated the pathological role of miR-425-5p in sepsis-induced AKI. The results suggested that miR-425-5p reduced LPS-induced inflammatory response through targeting TLR4 and then alleviated sepsis-induced AKI. Our data may offer novel evidence for investigating therapeutic target of AKI.

**Material and method**

**Septic mice induced**

The septic mice was induced followed previous description [[23](#_ENREF_23" \o "Rittirsch, 2009 #181)]. Thirty-six male C57BL/6 mice, 18-month-old, weighing 25‐30 g, were purchased from Experimental Animal Centre of Zhengzhou University and raised in specified pathogen-free (SPF) condition at 12h light/dark cycle. This study was permitted by the Animal Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Twelve mice were randomly assigned into sham and CLP group (n = 6, per group). Mice were anesthetized with isoflurane and then performed laparotomy. The peritoneum was cut longitudinally, and the cecum was separated and ligated. A perforation is made between the ligation and the apex of the cecum. After the needle is pulled, the fecal materials were squeezed into the peritoneal cavity. Mice were subcutaneously injected with 0.9% saline. The buprenorphine (0.05 mg/kg) was injected for postoperative analgesia. The mice in sham group were also performed laparotomy under isoflurane anesthesia, but not performed the ligation and puncture of cecum. For the miRNA agomir delivery, twenty-four mice were randomly grouped into sham, CLP, CLP + miR-425-5p agomir, and CLP + agomir-NC group (n = 6, per group). miR-425-5p agomir and its negative control agomir-NC were obtained from Ribobio Co., Ltd (Guangzhou, China), and dissolved in 0.9% saline and then injected into mice (100 μl, 10 nM/20 g body weight) through tail vein 24 h prior to CLP surgery [[24](#_ENREF_24" \o "He, 2020 #182)].

**Cell culture and transfection**

Human proximal tubular cells (HK-2 cells) were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, USA), 100 U/ml penicillin (Solarbio, Beijing, China) and 100 μg/ml streptomycin (Solarbio) in an incubator at 37℃ with 5% CO2. The miR-425-5p inhibitor, NC inhibitor, miR-425-5p mimic and NC mimic were designed by GenePharma Co., Ltd. (Shanghai, China). The overexpression plasmid vector of TLR4 (pcDNA-TLR4) and the control empty vector (pcDNA) were purchased from Ribobio Co., Ltd. (Guangzhou, China). All the transfections were performed by using Lipofectamine 2000 reagent (Invitrogen, USA) followed the producer's guidelines. After transfection for 24 h, LPS (2μg/ml) was added to cells and cultured for 24h to induce an in vitro septic model. Finally, cells were collected for follow-up detections.

**Quantitative real-time PCR (qRT-PCR)**

Total RNA of HK-2 cells and renal tissues of mice were isolated by using Trizol reagent (Invitrogen, USA). The purity and concentration of RNA were detected by a Nanodrop (Thermo Scientific, USA) with A260/A280 ratios from 1.9 to 2.1. Then, the RNA was reverse‐transcribed into complementary DNA (cDNA) by M-MLV Reverse Transcriptase Kit (Promega, USA). The qRT‐PCR was conducted with SYBR Green Master (Roche, Germany) according to the user's manual. U6 and β-actin were used as endogenous controls. The relative levels of molecules were estimated by the 2 −△△Ct method.

**Western blotting**

The HK-2 cells and renal tissues of mice were lysed in RIPA lysis buffer (Beyotime, Shanghai, China). The lysate was centrifugated at 15000 r/min, 4℃ for 15 min, and the supernatant was collected. The concentration of the protein was evaluated with the BCA Protein Assay Kit (Beyotime). Next, 20 μg protein samples were segregated by 10% SDS-PAGE and then transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Boston, MA). The membrane was blocked in 5% fat-free milk for 1 h at room temperature and then incubated with primary antibodies against anti-TLR4 (ab13556, Abcam, USA, 1:500) and anti-GAPDH (ab9485, Abcam, 1:2500) at 4°C overnight. Subsequently, the membrane was washed with PBST and incubated with secondary antibody goat anti-rabbit IgG (ab6721, Abcam, 1:3000) for 2h. The bands were visualized with ECL Plus Western Blotting Substrate (Thermo, USA).

**Enzyme linked immunosorbent assay (ELISA) and biochemical marker detection**

The cell supernatants in each group were centrifuged at 2000 rpm for10 min to remove cell debris. The serum of mice and cell supernatants were used for ELISA detection. The contents of TNF-α, IL-1β and IL-6 were measured by mouse and human ELISA Kit (R&D, USA) referred to the instructions. Scr and BUN levels in the serum of mice were detected to evaluate organ function by corresponding ELISA Kits (Nanjing Jiancheng, China). The absorbance at 450 nm was recorded by a microplate reader.

**Cell viability detection**

The cell viability was measured with a Cell Counting Kit-8 (CCK-8, New Jersey, USA). In brief, cells of 100 μL each well were seeded in 96-well plate for overnight, then the CCK-8 solution (10 μL) was added to cells and incubated for 3h in an incubator with 5% CO2 at 37℃. The absorbance at 450 nm was measured by a microplate reader (Bio-Rad, USA).

**Histological detection**

The renal tissues of mice were fixed with 4% paraformaldehyde overnight. Then, the specimens were processed with dehydration, embedded in paraffin, and sectioned into 4 µm thickness. The sections were stained with hematoxylin and eosin, and dehydrated. The sections were observed in light microscope. Six random fields were selected in each section and the representative images were captured.

**Dual luciferase reporter assay**

This assay was carried out followed the previous studies[[25](#_ENREF_25" \o "Xu, 2020 #197), [26](#_ENREF_26" \o "Niu, 2020 #198)]. Briefly, 2.5 × 105 293T cells were seeded in a 6-well plate a day before transfection. The wild type (WT) TLR4 3’-UTR (TLR4 3’-UTR-WT) and mutated (MUT) TLR4 3’-UTR (TLR4 3’-UTR-MUT) were inserted into the luciferase coding region in a pmirGLO plasmid. The plasmids and miR-425-5p mimic or NC mimic were co-transfected into 293T cells by Lipofectamine 2000. After that, the relative luciferase activity of TLR4 3’-UTR were assessed using a Dual-Luciferase® Reporter System (Promega, USA).

**Statistical analysis**

Statistical analysis was performed using the GraphPad Prism 7.0 software. Data were expressed as mean ± standard deviation. P<0.05 indicated the significant difference, and the group differences were compared by Student t test (two groups) and one-way ANOVA (multiple groups).

**Results**

**MiR-425-5p was down-regulated in CLP-induced septic mice.**

To investigate the expression of miR-425-5p in sepsis, the CLP-induced septic mouse model was established. Results in Figure 1A showed that the renal injury marker BUN and Scr were up-regulated in the serum of CLP-induced mice, illustrating the significant renal damage in CLP-induced mouse models. The serum concentrations of TNF-α, IL-1β and IL-6 were increased in CLP group compared with the sham group (Figure 1B-D). H&E staining was performed on renal tissues for pathological analysis. The histopathological damage in kidney was obviously seen in CLP group, which was characterized by marked tubular necrosis and vacuolar degeneration (Figure 1E). According to the result of DIANA tools software and GSE94717, and the screening conditions of FDR<0.05 and |logFC|>1, five miRNAs were obtained and detected in renal tissues (Figure S1). We found that the expressions of the five miRNAs were down-regulated in renal tissues of CLP-induced mice, and miR-425-5p expression reduced most significant compared with other miRNAs (Figure 1F). These data indicated that the CLP-induced mouse model was successfully established and miR-425-5p was down-regulated in septic mice.



**Figure1. MiR-425-5p was down-regulated in CLP-induced septic mice.** The septic mice were induced by CLP method, and the peripheral blood samples and renal tissues of mice were collected 24 h after the surgery. N = 6 per group. (A) The serum BUN and Scr levels of mice were measured. (B-D) The TNF-α, IL-1β and IL-6 levels were detected in the serum of mice by ELISA. (E) The pathological damage was examined. (F) The DIANA Tools software was used for screening mouse miRNAs that can regulate TLR4 signaling ([http://diana.imis.athena-innovation.gr/DianaTools/index.php](http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/results&keywords=ENSMUSG00000039005&genes=ENSMUSG00000039005%20&mirnas=&descr=&threshold=0.7)). The GSE94717 dataset was used to analyze the changed miRNAs in septic AKI patients. Using FDR<0.05 and |logFC|>1 as screening conditions, and five miRNAs (miR-141-3p, miR-425-5p, miR-374c-3p, miR-362-3p and miR-302a-5p) were obtained and displayed in a venn diagram. The expression of the obtained five miRNAs was detected in the renal tissues of septic mice. (\* P＜0.05, \*\*P＜0.01vs. sham group).



**Figure S1. Screening of miRNAs in renal tissues.** Venny map was constructed.

**Overexpression of miR-425-5p reduced production of pro-inflammatory cytokines in LPS-induced HK-2 cells.**

Next, we explored the role of miR-425-5p in vitro, miR-425-5p mimic (or NC mimic) was transfected into HK-2 cells and then treated with LPS. Compared with control group, the viability of HK-2 cells was decreased when treated with LPS, whereas miR-425-5p mimic transfection reversed this effect (Figure 2A). The TNF-α, IL-1β and IL-6 levels in cell supernatants were elevated, while these results were reversed by miR-425-5p mimic transfection (Figure 2B-D). Additionally, the expression of miR-425-5p in HK-2 cells was detected, and we discovered that miR-425-5p expression was reduced after treated with LPS, whereas miR-425-5p mimic transfection significantly enhanced the expression of miR-425-5p, implying that the transfection and the LPS treatment were effective. Collectively, these results implied that miR-425-5p was down-regulated in LPS-induced HK-2 cells, and miR-425-5p overexpression enhanced cell viability, and decreased production of pro-inflammatory cytokine.



**Figure2.** **MiR-425-5p was down-regulated in LPS-induced HK-2 cells.** The miR-425-5p mimic or mimic NC was transfected into HK-2 cells, followed by LPS (2 μg/ml) treatment for 24 h. Cells were grouped as follows: Control, LPS, LPS + miR-425-5p mimic, and LPS + mimic NC. After finishing the transfection and LPS treatment, cells and supernatants were collected for relative detection. (A) The cell viability was measured in each group by CCK8 assay. (B-D) The TNF-α, IL-1β and IL-6 levels in cell supernatants were measured by ELISA. (E) Expression of miR-425-5p in HK-2 cells was examined by qRT-PCR. Con: control; miR-425-5p: miR-425-5p mimic; NC: mimic NC. Data were from three independent experiments. (\*\*P＜0.01vs. LPS group, or LPS + NC group).

**MiR-425-5p reduced LPS-induced inflammatory response through targeting TLR4.**

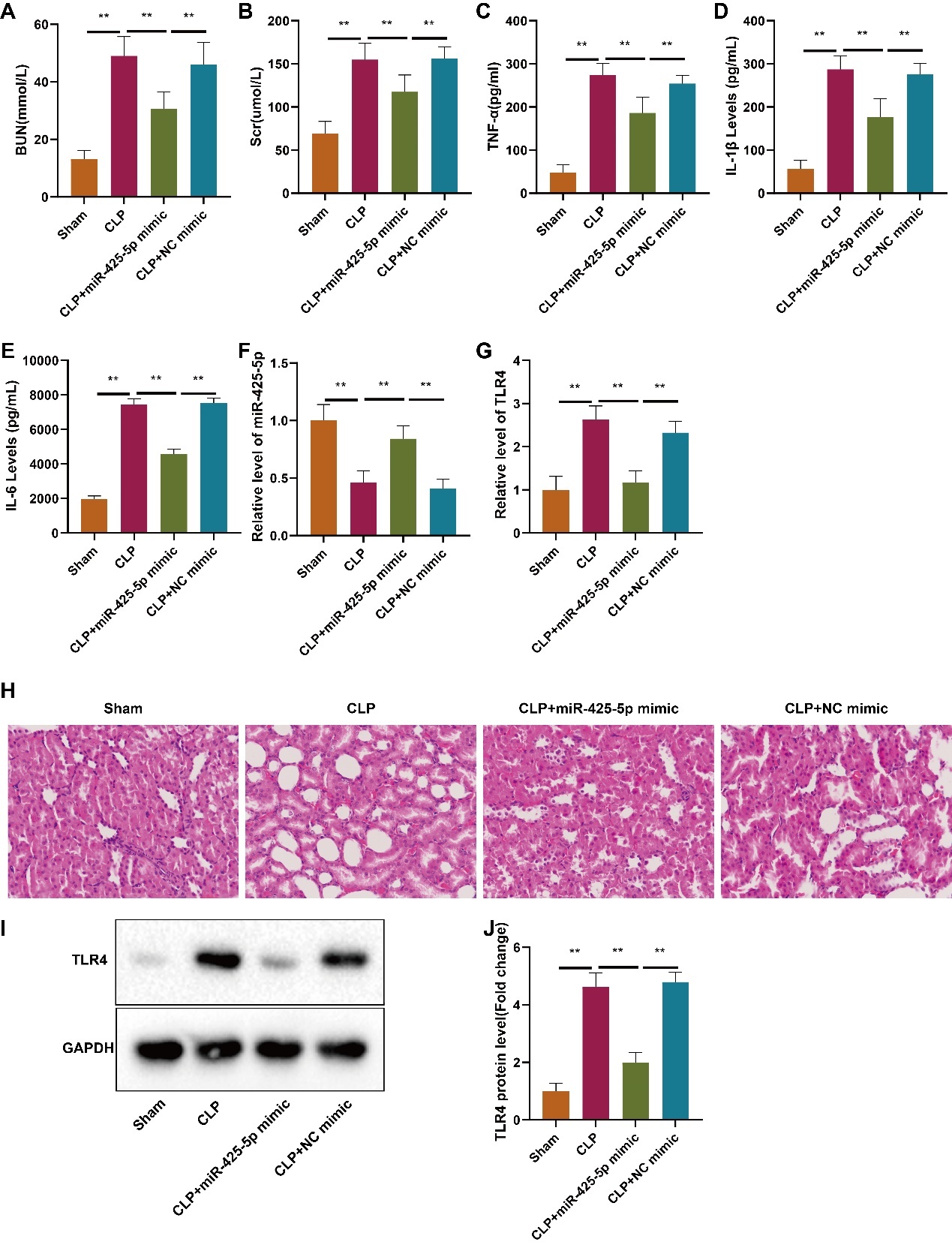
Given the importance of miR-425-5p in inflammation, we next determined its underlying mechanism. Through using the target gene predictive database, TLR4 was predicted as a probable target of miR-425-5p. The result conveyed that the 3 '-UTR of TLR4 contained the binding site for miR-425-5p (Figure 3A). Next, the luciferase reporter result displayed that co-transfection of the miR-425-5p mimic and TLR4 WT 3’-UTR significantly decreased the luciferase activity, while no significant change was observed in TLR4 MUT 3’-UTR or NC mimic transfection group (Figure 3B). Then, miR-425-5p mimic, miR-425-5p inhibitor and their negative controls were transfected into HK-2 cells. We discovered that miR-425-5p mimic transfection enhanced miR-425-5p expression, but reduced and the mRNA and protein level of TLR4, while miR-425-5p inhibitor transfection showed the opposite results (Figure 3C-3E). Next, the miR-425-5p mimic and pcDNA-TLR4 were co-transfected into HK-2 cells and then treated with LPS. ELISA results showed that the concentrations of TNF-α, IL-1β and IL-6 in cell supernatants were increased after transfected with miR-425-5p mimic, while co-transfection of miR-425-5p mimic and pcDNA-TLR4 reversed this result (Figure 3F-3H). Additionally, we found miR-425-5p mimic transfection enhanced the mRNA and protein level of TLR4, whereas pcDNA-TLR4 transfection reversed this effect (Figure 3I-3L). Taken together, these data conveyed that miR-425-5p decreased LPS-induced inflammatory response via targeting TLR4.



**Figure3. MiR-425-5p reduced LPS-induced inflammatory response through targeting TLR4.** (A)The target of miR-425-5p was predicted by using the targetscan and miRanda databases. The 3’ UTR of TLR4 showed the binging site of miR-425-5p. (B) miR-425-5p mimic and TLR4 3’-UTR (WT) were co-transfected into 293T cells. The NC mimic and the mutation of TLR4 3’-UTR (MUT) were the negative controls. Then, the luciferase activity was detected. (C-E) Next, the miR-425-5p mimic, miR-425-5p inhibitor and their negative controls were transfected into HK-2 cells. The miR-425-5p expression and TLR4 level were assessed. (F-H) The miR-425-5p mimic and pcDNA-TLR4 were co-transfected into HK-2 cells, followed by LPS (2 μg/ml) treatment for 24 h. Cells were assigned into 5 groups: Control, LPS+NC, LPS + miR-425-5p, LPS + miR-425-5p + TLR4, and LPS + miR-425-5p + NC. TNF-α, IL-1β and IL-6 levels in cell supernatants were measured by ELISA. (I-L) miR-425-5p expression, and TLR4 mRNA and protein levels were detected by qRT-PCR and western blot. Data were from three independent experiments. (\*\*P＜0.01, vs. NC mimic, or NC inhibitor, or LPS + miR-425-5p).

**Increased miR-425-5p expression ameliorated sepsis-induced AKI.**

We then determined whether miR-425-5p participated in the pathological progress *in vivo*.The miR-425-5p agomir (or agomir NC) was injected into mice through tail vein, and then subjected to CLP. As indicated in Figure 4A and 4B, the renal injury marker BUN and Scr levels in mouse serum were decreased in miR-425-5p agomir injection group compared with the agomir NC injection group. Similarly, miR-425-5p agomir injection reduced the serum levels of TNF-α, IL-1β and IL-6 (Figure 4C-4E). After injection of miR-425-5p agomir, the qRT-PCR results displayed that miR-425-5p expression in mouse renal tissues was elevated, while the mRNA level of TLR4 was decreased compared with agomir NC injection group (Figure 4F, 4G). H&E staining result showed that miR-425-5p agomir injection reduced the histopathological damage in renal tissue compared with the agomir NC injection group, which was characterized by lessened tubular necrosis, vacuolar degeneration and infiltration of inflammatory cells (Figure 4H). Besides, the protein level of TLR4 in renal tissues of mice was reduced after injection of miR-425-5p agomir (Figure 4I, 4J). Thus, overexpression of miR-425-5p could alleviate the inflammatory response and tissue damage in CLP-induced mouse models.



**Figure4. Overexpression of miR-425-5p alleviated AKI induced by CLP.** Mice were injected with miR-425-5p agomir or agomir NC through tail vein (30 mg/kg, body weight) for 3 consecutive days, followed by CLP surgery at 24 hours after the last agomir injection. The serum and renal tissues were collected. Mice were grouped (N = 6/group) into sham, CLP, CLP + miR-425-5p, and CLP + NC group. (A-B) The serum BUN and Scr levels were measured. (C-E) The concentrations of TNF-α, IL-1β and IL-6 in serum of mice were examined. (F-G) The expression of miR-425-5p and TLR4 were determined. (H) Representative pathological images of the H&E staining for kidney. (I-J) TLR4 protein level in renal tissues of mice was evaluated. miR-425-5p: miR-425-5p agomir; NC: agomir NC. (\*\*P＜0.01, vs. sham, or CLP + NC).

**Discussion**

Acute kidney injury (AKI) is a common complication of sepsis and is the leading cause of shock or death [[7](#_ENREF_7" \o "Zarbock, 2014 #8), [27](#_ENREF_27" \o "Kolling, 2018 #184)]. Although some progress has been made in recent years, it remains a big challenge for effective treatment to reduce mortality. Currently, increasing researches have realized the significance of inhibiting the sepsis-induced AKI via restraining the excessive immune response [[28](#_ENREF_28" \o "Funahashi, 2019 #185), [29](#_ENREF_29" \o "Zhang, 2020 #186)]. Furthermore, the high morbidity and mortality of elderly patients with sepsis suggests that it is urgent for us to explore the treatment of elderly patients with sepsis. In this study, we confirmed the effect of miR-425-5p overexpression against CLP-induced AKI in aging mice, and identified that miR-425-5p reduced inflammatory response via targeting TLR4. Thus, miR-425-5p is a possible therapeutic target for AKI.

Previous reports have claimed that miRNAs serve as gene expression switches in various processes of sepsis-induced AKI [[29-31](#_ENREF_29" \o "Zhang, 2020 #186)]. Such as, Shen et al. claimed that miR-106a targeted THBS2 gene to promote the production of pro-inflammatory cytokines, leading to aggravated renal injury of septic mice with AKI [[30](#_ENREF_30" \o "Shen, 2019 #187)]; Qin et al. demonstrated that miR-133a alleviated renal tissue damage in LPS-induced sepsis in vitro model by targeting BNIP3L [[31](#_ENREF_31" \o "Qin, 2020 #195)]; Zhang et al. showed that miR-20a restrained LPS-induced HK-2 cell injury via blocking inflammatory cytokine release [[29](#_ENREF_29" \o "Zhang, 2020 #186)]. On the basis of these evidences, we detected the miR-425-5p expression in this study. By using miRNA sequencing analysis with GEO database and DIANA Tools, we found five miRNAs were associated with sepsis-induced AKI patients and TLR4 signaling, and miR-425-5p expression was decreased in CLP-induced septic mice and LPS-induced HK-2 cells. Furthermore, we discovered that increased miR-425-5p expression alleviated renal injury in AKI, as evidenced by the improved histopathological damage, reduced pro-inflammatory cytokine production, and decline of BUN and Scr levels. Nevertheless, the underlying mechanism of miR-425-5p alleviates AKI is unclear.

TLR4 acts as an important role in promoting inflammation by producing inflammatory cytokines in AKI animal models, and increased TLR4 expression contributed to the aggravation of AKI [[32](#_ENREF_32" \o "Li, 2019 #190), [33](#_ENREF_33" \o "Zhong, 2020 #191)]. As reported before, TLR4 level was regulated by miRNAs in sepsis-related complications. For instance, Lv et al. confirmed that miR-27a was closely related to the inflammatory response via targeting TLR4 in septic cell model [[34](#_ENREF_34" \o "Lv, 2017 #192)]; Yuan et al. claimed that miR-201-5p protected against LPS-induced inflammatory response through inhibiting TLR4/NOTCH3 pathway [[35](#_ENREF_35" \o "Yuan, 2020 #193)]. Another study by Wang et al. discovered that miRNA-326 prevented sepsis-induced acute lung injury via targeting TLR4 [[21](#_ENREF_21" \o "Wang, 2020 #194)]. Therefore, we explored whether miR-425-5p exert protective role in sepsis-induced AKI via regulating TLR4. Our findings showed that TLR4 was a target of miR-425-5p, and miR-425-5p reduced TLR4 level through targeting the 3’-UTR of TLR4. Furthermore, increased TLR4 level abolished the effects of miR-425-5p overexpression on inflammation. These data suggest that miR-425-5p plays a crucial role in inflammatory response during sepsis-induced AKI through targeting TLR4.

In summary, the current study identified miR-425-5p expression was remarkably decreased in renal tissues of sepsis-induced AKI mice and LPS-treated HK-2 cells. Overexpression of miR-425-5p reduced inflammatory response and alleviated sepsis-induced AKI via targeting TLR4. This study conveyed the crucial role of miR-425-5p/TLR4 axis, which may offer novel insight for deeper discoveries of septic-AKI. Our research provides new insights into the development of useful drug therapies for clinical conversion in elderly patients with sepsis-induced AKI.

**DECLARATIONS**

**Acknowledgments**

Not applicable.

**Authors’ contributions**

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Hongbin Li, Xiaoxuan Niu, Xiaoge Sun;

Performed data acquisition, as well as provided administrative, technical, and material support: Dongyi jin, min gao, Dan Wei

**Availability of data and materials**

Not applicable.

**Financial support and sponsorship**

None.

**Conflicts of interest**

All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

This study was permitted by the Animal Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

**Consent for publication**

Not applicable.

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