The miR-15a-5p-XIST-CUL3 regulatory axis is important for sepsis-induced acute kidney injury

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**ABSTRACT**

**Background:** Acute kidney injury (AKI) refers to a sudden loss of renal function. This study was performed to identify the key RNAs acting in the mechanism of sepsis-induced AKI.

**Methods:** Microarray dataset GSE94717 (including six sepsis-induced AKI samples and three control samples) was downloaded from Gene Expression Omnibus database. Differentially expressed miRNAs (DE-miRNAs) were identified. The miRNA targets were predicted and enrichment analysis was performed. Protein-protein interaction (PPI) and competing endogenous RNA (ceRNA) regulatory networks were constructed. Mouse podocytes were treated with lipopolysaccharide (LPS), following by cell viability and PCR analysis. Cellular apoptosis and the ceRNA network were validated.

**Results:** Thirty-one common DE-miRNAs (two up-regulated and 29 down-regulated) by AKI versus control and male AKI versus control were identified. We found the targets of miR-15a-5p, miR-15b-5p, and miR-16-5p were involved in mTOR signaling pathway, and those of miR-29b-3p and miR-16-5p were enriched in PI3K-Akt signaling pathway. RNAs including miR-15b-5p, miR-15a-5p, miR-107, XIST, miR-16-5p, and cullin3 gene (CUL3) were included in the ceRNA regulatory network. The downregulation of miR-15a-5p and miR-15b-5p and the upregulation of lncRNA XIST and CUL3 gene were validated using qPCR. The miR-15a-5p-XIST-CUL3 regulatory axis was identified and was validated. We confirmed that LPS inhibited the growth of mouse podocytes and seven of the ten miRNAs, but upregulated XIST and CUL3. Transfection analysis showed XIST siRNA enhanced LPS-induced MPC5 cell apoptosis and miR-15a-5p inhibitor reserved it, so did as CUL3 overexpression for miR-15a-5p mimics.

**Conclusion:** The miR-15a-5p-XIST-CUL3 regulatory axis was related to the pathogenesis of sepsis-induced AKI.

**HIGHLIGHTS**

- Totally, 31 miRNAs were dysregulated between disease and control groups.
- miR-15a-5p, miR-15b-5p, and miR-16-5p were involved in mTOR signaling pathway.
- miR-16-5p and miR-29b-3p were implicated in PI3K-Akt signaling pathway.
- The miR-15a-5p-XIST-CUL3 axis was critical for sepsis-induced AKI.

**Introduction**

Acute kidney injury (AKI) is a sudden loss of renal function that happens in less than seven days [1]. It can be induced by various processes, and the most common causes are dehydration, sepsis, and nephrotoxic drugs [2,3]. AKI is usually diagnosed based on increased blood and urea nitrogen, or insufficient urine produced by the kidneys [4]. The complications of AKI include high potassium content, metabolic acidosis, variation of fluid balance, and uremia [5]. Sepsis-induced AKI occurs in 40% patients with sepsis, namely septic AKI [6]. Recent studies demonstrated that patients with AKI may have...
increased renal blood flow, tubular injury but not necrosis and apoptosis [6,7]. Therefore, the mechanisms of AKI are complex and should be deeply explored to improve the therapies of the disease.

MicroRNA (miRNA) can cause gene silencing by binding mRNA, while competing endogenous RNA (ceRNA, such as long non-coding RNA (lncRNA), mRNA, circular RNA (circRNA), pseudogene transcript, et al) can mediate gene expression by competitively binding miRNA using microRNA response elements (MREs) [8,9]. For instance, Sirtuin 3 (SIRT3) protects against mitochondrial damage in sepsis-induced AKI through suppressing the NLR family, pyrin domain-containing 3 (NLRP3) inflammasome, reducing reactive oxygen species (ROS) production, decreasing interleukin-1β (IL1B) and IL18 expression, and weakening oxidative stress [10,11]. LncRNA HOXD transcript antisense RNA (HOTAIR) has a high expression in sepsis-induced AKI, which facilitates the apoptosis of HK-2 cells in AKI via the miR-22/high mobility group box 1 (HMGB1) pathway [12]. Through repressing c-Jun N-terminal kinase (UNK)/ nuclear factor-κB (NF-κB) pathway and binding to tumor necrosis factor-α (TNF-α), lncRNA plasmacytoma variant translocation 1 (PVT1) may enhance inflammatory response in lipopolysaccharide (LPS)-induced septic AKI [13]. In addition, overexpressed lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) may worsen AKI via stimulating NF-κB pathway and regulating miR-204, therefore, NEAT1 may have impressive roles in sepsis-induced AKI [14]. Although the above studies have explored the RNAs involved in sepsis-induced AKI, the pathogenesis of this disease have not been entirely reported.

In the current study, the miRNA expression profile of sepsis-induced AKI was downloaded and analyzed. Through differential expression analysis, miRNA-target prediction, enrichment analysis, and network analysis, the crucial RNAs and ceRNA regulatory relationships were identified in sepsis-induced AKI. This study might further reveal the pathogenesis of the disease and provide theoretical support for its clinical treatment.

Materials and methods

Microarray data

The miRNA microarray dataset, under GSE94717 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94717; platform: GPL19449Exiqon miRCURY LNA microRNA Array, 7th generation REV-hsa, mmu & rno (miRBase v18.0)] was downloaded from Gene Expression Omnibus (GEO) database. GSE94717 consisted of 15 blood samples collected from 6 patients with G-sepsis-induced AKI, 6 patients with G-sepsis-non AKI and 3 healthy controls. The data from samples from patients with G-sepsis-induced AKI (n = 6, mean age = 64.17 years old, two females and four males) and from healthy controls (n = 3, mean age = 60 years old, two females and one male) were selected for the further analysis.

Data preprocessing and differential expression analysis

The matrix data of the microarray dataset was acquired and subjected to background correction and normalization using the R package limma (version3.10.3, http://www.bioconductor.org/packages/2.9/bioc/html/limma.html) [15]. Subsequently, the expression matrix was divided into sepsis-induced AKI group and control group, and the significance of p values of expression difference was calculated based on the unpaired t-test in limma package [15]. The differentially expressed miRNAs (DE-miRNAs) were identified with the thresholds of |log fold change (FC)| ≥ 1 and p values < .01.

miRNA-target prediction and enrichment analysis

The target genes of the DE-miRNAs were predicted using the miRWalk2.0 tool [16] (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/). To ensure the accuracy of target prediction, the miRNA-target pairs included in at least 7 of miRWalk, miRanda, miRDB, miRMap, miRBase, RNA22, TargetsCan, and mirbridge databases were screened. The miRNA-target regulatory network was constructed using Cytoscape software (version 3.2.0, http://www.cytoscape.org) [17].

Bioinformatic enrichment was performed for the genes included in the miRNA-gene pairs. Gene Ontology (GO), including cellular component (CC), biological process (BP), and molecular function (MF) categories [18] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [19] enrichment was conducted combined with DAVID online tool [20] (version 6.8; https://david-d.ncifcrf.gov/). Meanwhile, the R package clusterProfiler [21] (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html) was utilized to perform KEGG enrichment analysis for the miRNAs in the miRNA-target regulatory network with the number of target genes ranked in the top 10. The significant thresholds for selecting the results of enrichment analysis were set as gene count ≥ 2 and p values < .05.
Protein-protein interaction (PPI) network analysis for the target genes

The interactions among the genetic productions of the targets were identified in STRING database [22] (version 10.0; http://string-db.org/; combined score > 0.4). PPI network was visualized using the Cytoscape software [17]. To obtain the key target genes, the network topology property index Degree Centrality was used to analyze the scores of network nodes. The higher the node score, the more important the location of the node was in the network. The significant network modules were screened using the MCODE plug-in [23] in Cytoscape software, with the threshold of score > 5.

CeRNA regulatory network analysis

The miRNA-lncRNA pairs involving the DE-miRNAs were screened in starBase database [24] (version 2.0, http://starbase.sysu.edu.cn/), with the thresholds of low stringency ≥ 1 and number of cancer types ≥ 1. The lncRNA and mRNA regulated by the same miRNAs were screened from the miRNA-mRNA pairs and miRNA-lncRNA pairs, namely the miRNA-lncRNA-mRNA or ceRNA pairs. ceRNA regulatory network was visualized using Cytoscape software [17].

Patient collection

A total of five patients (male = 4 and female = 1, aged 45.6 ± 6.9 years old) were collected from Department of ICU, the first people’s hospital of Xiaoshan District, Hangzhou, during February 2019 to July 2019. Five sex- and age-matched healthy controls (47.1 ± 8.2 years old), without known diseases, were collected from our hospital. The fasting peripheral blood samples were collected from all patients and healthy controls. Blood samples were prepared and RNA was isolated and stored at −20°C before analysis. The human experiments were approved by the Ethics Committee of the First People’s Hospital of Xiaoshan District, Hangzhou. Written informed consents were obtained from all participants before blood sampling.

Cells and LPS induction

Mouse podocytes (MPCs) were purchased from the Cell Bank of the Chinese academy of sciences (Shanghai, China). Cells were maintained in RPMI-1640 (Invitrogen, Shanghai, China) supplemented with 10% FBS (Invitrogen) at 37°C, 5% CO2. MPCs were treated with 100 ng/mL LPS (Sigma-Aldrich) for 48 h [25].

Cell proliferation assay

Cell viability was tested using the Cell Counting Kit 8 (CCK8) assay kit (Beyotime, Shanghai, China). Cells were harvested at 0, 12, 24, and 48 h post LPS induction and then incubated in CCK8 solution for 2 h. Cell viability was detected using a microplate reader (Bio-Rad Labs, Sunnyvale, CA) and the optical density at 450 nm was detected. Each experiment was performed in triplicate.

Dual-luciferase reporter assay

The interactions between IncRNA and miRNA and between miRNA and target were predicted using the LncBase Predicted v(0).2 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php) and miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/php/detail.php), respectively. The interaction was validated using dual-luciferase reporter system. The luciferase vectors containing wild type (WT) and mutant (MUT) binding sites of miRNAs in the 3’ UTR regions of XIST and DUL3 genes were constructed using psiCHECK-2 expression vector (Promega, USA) [26]. Cell transfection into MPC5 cells was performed using Lipofectamine 2000 regents (Invitrogen), following the manufacturer’s instructions.

Cell transfection

MPCs (1 × 10^5 cells/well) were seeded into 24-well plates, and then transfected with siRNAs targeting XIST, miR15b-5p mimics, inhibitors, and the scramble sequences (NC; GenePharma, Shanghai, China) for 6 h. For the overexpression of CUL3 gene, cells were transfected with CUL3-overexpressing (OE-CUL3) plasmids constructed by cloning the full length of human CUL3 gene coding region into pcDNA3.1 vectors (Genechem). Empty pcDNA3.1 was used as control for OE-CUL3 transfection. Cell transfections were conducted using the Lipofectamine 2000 (Invitrogen) according to the manufacturers’ instruction. Then MPC5 cells were treated with 100 ng/mL LPS (Sigma-Aldrich) for 48 h. Cell proliferation was detected after LPS treatment.

Cellular apoptosis

LPS-induced MPC5 cell apoptosis was detected using Annexin V/PI double staining (BD Biosciences, San Jose, CA, USA). Transfected cells (5 × 10^5 cells/ml) were placed into 6-well plates and then treated with LPS as previously reported. Cells were harvested and then digested into single-cell suspensions, which were then incubated with Annexin V-FITC/PI staining solutions (BD
and then detected using a BD FACS Calibur™ flow cytometry (BD Biosciences). Each experiment was performed in triplicate.

**Real-time PCR analysis**

Cellular and blood RNAs were extracted using TRIzol reagent (Invitrogen). Reverse transcription of miRNA and mRNA was performed with reverse transcription and a DBI Bestar qPCR RT Kit (DBI Bioscience, Shanghai, China), respectively, following the manufacturer's instructions. Primers were synthesized by Sangon (Shanghai, China; Table 1). Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was employed for the PCR amplification. GAPDH was used as the reference gene for mRNA and lncRNA, and U6 for miRNA, respectively. The relative expression level of each RNA was calculated using $2^{-\Delta\Delta Ct}$ methods.

**Statistical analyses**

All data are expressed as the mean ± standard deviation (SD). Student’s $t$-test was used for differences between groups. A $p$ value < .05 was considered statistically significant.

**Results**

**Differential expression analysis**

We firstly identified the DE-miRNAs from male patients ($n = 4$), and 51 DE-miRNAs, including 10 up-regulated and a DBI Bestar qPCR RT Kit (DBI Bioscience, Shanghai, China), respectively, following the manufacturer’s instructions. Primers were synthesized by Sangon (Shanghai, China; Table 1). Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was employed for the PCR amplification. GAPDH was used as the reference gene for mRNA and lncRNA, and U6 for miRNA, respectively. The relative expression level of each RNA was calculated using $2^{-\Delta\Delta Ct}$ methods.

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**Table 1.** The sequences of the used PCR primers.

| Gene    | Primers  | Sequences (5’–3’)         |
|---------|----------|---------------------------|
| miR-15a-5p | Forward  | TAGCACCACTATGTTG          |
| miR-92a-3p | Forward  | TACCTACTTTGCTGG           |
| miR-15b-5p | Forward  | TAGCACCACTATGTTG          |
| miR-107  | Forward  | AGCCACATTGTACAGG           |
| miR-16-5p | Forward  | TACCTACTTTGCTGG           |
| miR-19b-3p | Forward  | TGGCGAATTCATGAAAATGG       |
| miR-29b-3p | Forward  | TGGCGAATTCATGAAAATGG       |
| miR-19a-3p | Forward  | TGGCGAATTCATGAAAATGG       |
| miR-144-3p | Forward  | TGGCGAATTCATGAAAATGG       |
| For all miRNA | Reverse  | CTCAACTGGGTCGTTG          |
| U6      | Forward  | CTGCCTTGGCAACCA           |
| GAPDH   | Reverse  | GCACCTGCAAGCTGG           |
| XIST    | Forward  | GCTCCACAAATCTAAG          |
| CUL3    | Reverse  | AGATGTTTACGAGCACATC       |
| Reverse  | ATGTCTTGTTGCTGG          |

**Figure 1.** The Venn figure and bidirectional clustering heatmap for the differentially expressed miRNAs (DE-miRNAs). (A) The Venn figure of the DE-miRNAs between the whole AKI patients versus healthy controls and male AKI patients versus healthy controls; (B) The bidirectional clustering heatmap.
and 41 down-regulated DE-miRNAs were identified versus controls (Figure 1(A)). With the mixing of two female samples (whole), 31 DE-miRNAs (two up-regulated and 29 down-regulated) were identified in sepsis-induced AKI group (male = 4, female = 2) compared with control group (male = 1, female = 2; Figure 1(A)), and all the 31 DE-miRNAs were common DE-miRNAs between the two comparisons. The bidirectional clustering heatmap of 31 common DE-miRNAs could clearly distinguish the samples in different groups (Figure 1(B)). We then focused on the function of the 31 common DE-miRNAs.

**MiRNA-target prediction and enrichment analysis**

A total of 564 miRNA-target pairs (involving 17 down-regulated miRNAs) were predicted (Figure 2). The top 10 miRNAs with higher interaction degrees in the regulatory network are listed in Table 2. Enrichment analysis showed that genes in the miRNA-target pairs were enriched into 111 GO_BP (such as positive regulation of transcription from RNA polymerase II promoter), 33 GO_CC (such as cytoplasm), and 43 GO_MF (such as protein binding), and 19 KEGG pathways (such as focal adhesion). The top 20 terms in each category are presented in Figure 3. Clusterprofiler

![Figure 2. The miRNA-target regulatory network. Circles and arrows represent down-regulated miRNAs and target genes, respectively.](image)
analysis showed the top 10 miRNAs (down-regulated) were associated with 41 KEGG pathways, and the top 5 pathways enriched for each miRNA are shown in Figure 4. Especially, miR-15a-5p, miR-15b-5p, and miR-16-5p were involved in mTOR signaling pathway, and miR-16-5p and miR-29b-3p were enriched in PI3K-Akt signaling pathway.

Table 2. The top 10 miRNAs in the miRNA-target regulatory network.

| miRNA       | Description | Degree |
|-------------|-------------|--------|
| hsa-miR-15a-5p | down        | 71     |
| hsa-miR-92a-3p | down        | 71     |
| hsa-miR-15b-5p | down        | 69     |
| hsa-miR-107   | down        | 53     |
| hsa-miR-16-5p | down        | 43     |
| hsa-miR-19b-3p| down        | 42     |
| hsa-miR-29b-3p| down        | 42     |
| hsa-miR-19a-3p| down        | 34     |
| hsa-miR-513a-5p| down       | 32     |
| hsa-miR-144-3p | down        | 31     |

PPI network analysis for the target genes

The PPI network for the target genes included 281 nodes (productions) and 776 edges (interactions; Figure 5). Three significant network modules (module A, score = 15, involving 15 nodes and 105 edges; module B, score = 8.2, involving 11 nodes and 41 edges; module C, score = 6, involving 6 nodes and 15 edges) were identified (Figure 6). The top 10 nodes in the PPI network and the nodes in the three significant network modules are listed in Table 3.

CeRNA regulatory network analysis

For the top 10 miRNAs in the miRNA-target regulatory network, 38 miRNA-lncRNA pairs were predicted. After integrating the above mentioned miRNA-lncRNA pairs and the miRNA-target pairs, 89 miRNA-lncRNA-mRNA regulatory pairs (involving 10 down-regulated miRNAs, 15 lncRNAs, and 28 mRNAs) were obtained (Figure 7),
and the nodes with top 10 degrees (including miR-29b-3p; miR-15b-5p; miR-15a-5p; miR-107; X inactive specific transcript, XIST; miR-19a-3p; miR-16-5p; miR-19b-3p; miR-92a-3p; and Cullin 3, CUL3) are listed in Table 4. Importantly, the miR-15b-5p/miR-16-5p/miR-19a-3p/miR-19b-3p/miR-92a-3p/miR-107-XIST-CUL3 regulatory axis involving the top 10 nodes existed in the ceRNA regulatory network.

Validation of the downregulation of RNA expression in AKI samples and in vitro LPS-induced cellular AKI model

The validation using AKI samples confirmed the significant downregulations of miR-15a-5p, miR-15b-5p, miR-107, miR-29b-3p and the upregulation of XIST and CUL3 (Figure 8(A)). The downregulation of miR-92a-3p, miR-16-5p, miR-19a-3p, and miR-144-3p were not significant in AKI samples versus controls (p > .05). In the in vitro validation experiments in MPC5, we found LPS treatment significantly decreased the relative expression of 7 of 9 miRNAs, including miR-92a-3p, miR-15a-5p, miR-107, miR-16-5p, miR-29a-3p, miR-19a-3p and miR-144-3p (p < .05, Figure 8(B)), and inhibited the proliferation of MPC5 (p < .01, Figure 8(C)). The expression of XIST and CUL3 were obviously upregulated by LPS (p < .01).

The confirmation of XIST-miR-15a-5p-CUL3 ceRNA axis

The interactions of XIST/miR-15a-5p and miR-15a-5p/CUL3 were predicted and then validated using the dual-luciferase reporter assay systems (Figure 9(A,B)). This suggested the dysregulated miR-15a-5p-XIST-CUL3 ceRNA axis during AKI. The apoptosis analysis using the transfected MPC5 cells showed that the inhibition of XIST and miR-15a-5p respectively enhanced and reserved LPS-induced apoptosis significantly (Figure 10(A) and Figure S1), while
Figure 5. The protein-protein interaction (PPI) network for the target genes. The higher the degree value, the larger the node.

Figure 6. The significant modules identified from the protein-protein interaction (PPI) network. (A) The significant module A; (B) The significant module B; (C) The significant module C.
the miR-15a-5p inhibitor reversed XIST siRNA enhanced MPC5 cell apoptosis. In addition, the overexpression of CUL3 significantly reduced LPS-induced apoptosis, and showed inhibitory effect on miR-15a-5p mimics induced apoptosis (Figure 10(B)). These data revealed the XIST-miR-15a-5p-CUL3 axis.

**Discussion**

In this study, 31 DE-miRNAs (two up-regulated and 29 down-regulated) were identified in blood samples from patients (two females and four males) with sepsis-induced AKI compared with healthy controls. This was different from the 37 DE-miRNAs by Ge et al by
compared with sepsis-induced AKI and sepsis-non AKI [27]. However, some miRNAs were also identified to be downregulated in sepsis-induced AKI versus sepsis-non AKI, like miR-15b-5p, miR-15a-5p and miR-16-5p. We identified several key miRNAs, including miR-15a-5p, miR-15b-5p, and miR-16-5p were involved in mTOR signaling pathway, and miR-16-5p and miR-29b-3p were associated with PI3K-Akt signaling pathway, the similar pathways identified by Ge et al. These two pathways have been reported to be impressive in ischemia-reperfusion (I/R)-induced AKI [28]. Additionally, miR-29b-3p, miR-15b-5p, miR-15a-5p, miR-107, XIST, miR-19a-3p, miR-16-5p, miR-19b-3p, miR-92a-3p, and CUL3 were the top 10 nodes in the ceRNA regulatory network. Therefore, the significant downregulation of these miRNAs might suggest the important roles of them in sepsis-induced AKI via involving mTOR and PI3K-Akt signaling pathways.

The overexpression of miR-29a was found to be independent risk factor for the mortality of patients with septic AKI, and thus they can be applied for predicting the 28-day mortality of the disease [29]. Huo et al reported that the expression level of miR-29a was positively correlated with the levels of serum creatinine, cystatin C, and kidney injury molecule-1 (KIM-1) in patients with septic AKI. However, Drummond et al reported that miR-29a-3p was increased in the kidney of mice exposed to e-cigarette air. The expression of its targets including Collagen 1A1 and Fibrillin 1 as well as renal fibrosis was significantly increased [30]. Overexpressed miR-107

Table 4. The top 10 nodes in the competing endogenous RNA (ceRNA) regulatory network.

| Nodes      | Description | Degree |
|------------|-------------|--------|
| hsa-miR-29b-3p | miRNA       | 16     |
| hsa-miR-15b-5p | miRNA       | 13     |
| hsa-miR-15a-5p | miRNA       | 12     |
| hsa-miR-107   | miRNA       | 10     |
| XIST         | lncRNA      | 10     |
| hsa-miR-19a-3p | miRNA       | 8      |
| hsa-miR-16-5p | miRNA       | 10     |
| hsa-miR-19b-3p | miRNA       | 7      |
| hsa-miR-92a-3p | miRNA       | 7      |
| CUL3          | gene        | 5      |

lncRNA: long non-coding RNA.

Figure 8. The validation experiments. A and B, the qPCR analysis for several differentially expressed miRNAs in five patients with AKI (male = 4 and female = 1) versus healthy controls (sex and age-matched), and that in LPS-induced cellular AKI model. C, the cell viability by CCK8 assay in MPC5. *p < .05 vs. blank. **p < .01 vs. blank. LPS: lipopolysaccharide.

Figure 9. The results of dual-luciferase reporter assay. The interaction between lncRNA and miRNA (A) and between miRNA and target (B) were predicted using the LncBase Predicted v(0).2 and miRTarBase, respectively. The interaction was validated using dual-luciferase reporter system. **p < .01 vs. NC.
causes TNF-α secretion through regulating dual-specificity phosphatase 7 (DUSP7) in circulating endothelial cells, which may lead to tubular cell damage in sepsis-induced AKI [31]. Our present study demonstrated that the DUSP3 is a predictive target of miR-107 and was upregulated by LPS treatment in MPC5. CUL3 is part of the ubiquitin proteasomal system and the CUL3 disruption is associated with tubulointerstitial fibrosis [32]. Saritas et al reported that the upregulation of CUL3 in patients with AKI and Cul3 disruption increased the expression of cyclin E and p21 and the promotion of proximal tubule injury in mice [32]. In addition, other studies identified the elevation of miR-92a-3p in chronic kidney injury (CKD) and CKD-associated atherosclerosis [33,34]. These suggested that miR-29b-3p, miR-107, and miR-92a-3p might be correlated with the pathogenesis of sepsis-induced AKI. The specific mechanism mediated by them should be explored.

The downregulation of miR-15a-5p has been identified in CKD with hypertension [35,36]. In addition, Ge et al suggested the downregulation of miR-16-5p, miR-15a-5p and miR-15b-5p in sepsis-induced AKI versus sepsis non AKI [27]. This consistency showed the important roles of these miRNAs in AKI. The difference is our present study identified lncRNAs related to these miRNAs. The expression of lncRNAs XIST and NEAT1 are significantly increased in glomerular and tubular epithelial cells, and urinary XIST serves as a potential marker for detecting membranous nephropathy [37]. Elevated XIST, which could be induced by LPS, is correlated with glomerular nephritis [37]. Our in vitro experiment using LPS-treated MPC5 cells showed XIST was elevated by LPS stimulus. We identified XIST regulated the expression of upregulated CUL3 by sponging the top 10 downregulated miRNAs, including miR-15a-5p, miR-16-5p and miR-107. The upregulation of CUL3 has been identified in patients with AKI [32]. Mutations in CUL3 reduced ubiquitination in the kidneys [38,39]. These studies indicated that XIST plays impressive roles in AKI via regulating CUL3 by sponging miRNAs. The miR-15a-5p-XIST-CUL3 regulatory axis in the ceRNA regulatory network was validated using the in vitro cellular experiment. We also demonstrated that miR-15a-5p inhibition reserved MPC5 cell apoptosis that was enhanced by XIST siRNA, so did as the overexpression of CUL3 for miR-15a-5p mimics-induced apoptosis in MPC5 cells. These data suggested that miR-15a-5p-XIST-CUL3 played crucial roles in sepsis-induced AKI, and the management targeting this axis might be of great value for controlling AKI development.

**Conclusions**

In conclusion, we identified 31 DE-miRNAs in sepsis-induced AKI samples compared with healthy control. Among them, miR-29b-3p, miR-15b-5p, miR-15a-5p, miR-107, XIST, miR-19a-3p, miR-16-5p, miR-19b-3p, miR-92a-3p, and CUL3 were closely related to the pathogenesis of sepsis-induced AKI. Moreover, the miR-15a-5p-XIST-CUL3 ceRNA regulatory axis was involved in the mechanism of sepsis-induced AKI and the LPS-induced injury in MPC5 cells.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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