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LINC00520 targeting miR-27b-3p regulates *OSMR* expression level to promote acute kidney injury development through the PI3K/AKT signaling pathway

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Abstract

Background: Acute kidney injury (AKI) shows several kinds of disorders, which acutely harm the kidney. However, the current medical methods have limited therapeutic effects. The present study aimed to find out the molecular mechanism of AKI pathogenesis, which may provide new insights for future therapy.

Methods: Bioinformatic analysis was conducted using the R language (AT&T BellLaboratories, University of Auckland, New Zealand) to acquire the differentially expressed long noncoding RNAs (lncRNAs) and messenger RNAs (mRNAs) in AKI. The expression levels of RNAs and related proteins in tissues and cells were detected by quantitative real-time PCR (qRT-PCR) and western blot. Dual-luciferase reporter gene assays were performed to verify the target relationship between microRNA (miRNA) and lncRNA as well as miRNA and mRNA. Flow cytometry and tunnel assay were used to detect the cell apoptotic rate in AKI.

Results: LINC00520, miR-27b-3p, and *OSMR* form an axis to regulate AKI. Knockdown of LINC00520 reduced acute renal injury both in vitro and in vivo. LINC00520 activated the PI3K/AKT pathway to aggravate renal ischemia/reperfusion injury, while upregulation of miR-27b-3p or downregulation of *OSMR* could accelerate the recovery of AKI.

Conclusion: Overexpression of LINC00520 contributes to the aggravation of AKI by targeting miR-27b-3p/*OSMR*.

KEYWORDS
acute kidney injury, LINC00520, miR-27b-3p, *OSMR*, PI3K-AKT

1 | INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome characterized by the rapid decline of renal function. The occurrence of AKI is associated with many conditions, and it occurs in hospitalized patients, particularly in those with multiorgan failures (L. Liu et al., 2018; Vanmassenhove, Kielstein, Jorres, & Biesen, 2017; Zuk & Bonventre, 2016). AKI is increasingly prevalent in both developing and developed countries, according to an epidemiology study (X. Xu et al., 2015). The reported mortality rate of AKI in hospitalized adults ranges from 14% to 60% (Chang et al., 2015). The long-term risk of AKI mortality, including chronic kidney disease (CKD), end-stage renal disease (ESRD), congestive heart failure (CHF), acute myocardial infarction, and stroke, has been studied before (Coca, Singanamala, & Parikh, 2012; Odutayo et al., 2017). Given that dialysis and continuous renal replacement therapy are common treatment methods in the clinic, which might bring huge physical damage to patients, new therapeutic methods need to be

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developed. Therapeutic approaches that effectively mitigate AKI could therefore also potentially have a major beneficial impact on long-term kidney graft function after transplantation. However, although animal models have provided detailed mechanistic insights into the pathophysiology of AKI, to date, no protective clinically applicable therapies to counteract AKI are available (van Zonneveld, Rabelink, & Bijkerk, 2017). It is important to explore more therapeutic strategies for AKI.

Numerous studies have focused on microRNAs (miRNAs) as biomarkers for the detection and prognosis of AKI, which indicates that noncoding RNAs (ncRNAs) might be a potential biomarker for AKI (Zou & Zhang, 2018). More than 80% of the human genes are transcribed into RNAs with no potential of protein-coding (Lorenzen et al., 2015). These so-called ncRNAs are arbitrarily separated into long noncoding RNAs (lncRNAs; >200 nucleotides); small ncRNAs (<200 nucleotides); and miRNAs (~22 nucleotides) on the basis of their size (ENCODE Project Consortium, 2012). In addition, ncRNAs can also be classified according to their function or subcellular localization such as ribosomal (rRNAs), transfer RNAs (tRNAs), small nucleolar RNAs (snoRNAs), short interfering RNAs (siRNAs), and so on (Zhou, Chen, Zou, & Wan, 2016). These noncoding RNAs can determine cell fate through the regulation of gene expressions, and repress many genes at the same time to directly influence the output of functionally related biological pathways and consequently, cell fate (Tamer, Polat, Yucebilgic, Guvenc, & Baslamisli, 2000). Recently, miRNA has been studied in numerous aspects, such as human cytomegalovirus (X. He, Teng, Cui, Li, & Wen, 2018), cancers (Lv et al., 2018; Pollard et al., 2018), and organ fibrosis (Chen et al., 2018; Tang et al., 2018). It was indicated that miRNAs could control the expression of multiple proteins through hundreds or thousands of targets influencing multiple signaling pathways (Zou & Zhang, 2018). Given that fewer studies focused on the functions of miRNAs in AKI progression, our study focuses on this aspect.

lncRNAs have also been studied deeper than usual in recent years, and it has been found that they can regulate the growth of tumors (Ding et al., 2018; T. Xu et al., 2018). Among the multiple working mechanisms of lncRNAs, the competitive endogenous RNA (ceRNA) theory was first proposed by Salmena and became prevalent and widely accepted (Wang et al., 2018). Recent studies presented emerging evidence that lncRNAs are vital regulators in human diseases, including AKI (Yu et al., 2016). Furthermore, microarray assays and RNA sequencing have been used to analyze lncRNA expressions in animal models of I/R (ischemia/reperfusion) injury or AKI patients, and some lncRNAs have been found to influence AKI (Zhou et al., 2016). However, the mechanism of lncRNA modulating the expression and biological functions of microRNA in AKI remains unclear. Thus, this study focuses on the mechanism of AKI accompanied by lncRNA and miRNA.

Oncostatin M (OSM) is a secreted cytokine mainly involved in chronic inflammatory and cardiovascular diseases through binding to OSM receptor β (*OSMR;* Plesner & Plesner, 1988). *OSMR* encodes a member of the type I cytokine receptor family. It was reported that disruption of the *OSMR* leads to the development of both mature-onset obesity and systemic insulin resistance by regulating the function of macrophages in mice (Komori et al, 2013, 2014). Besides, upregulation of the *OSMR* in cervical squamous cell carcinoma cells is associated with a proangiogenic phenotype and increased cell motility (Winder et al., 2011). Furthermore, the silencing of *EBLN1* induces cell apoptosis of Human Oligodendroglia cells, which may result from upregulation of *OSMR* (P. He et al., 2016). In addition, Luyckx, Cairo, Compston, Phan, and Mueller (2009) showed that the kidney can generate a strong acute phase response, which could be mediated by the OSM/OSMR. However, the roles of *OSMR* as well as lncRNA/miRNA/*OSMR* axis in the development and progression of AKI remain unclear.

In our current study, we focused on investigating the regulatory effect of the lncRNA and related miRNA on the progression of AKI and figuring out the specific mechanisms of AKI progression.

2 | MATERIALS AND METHODS

2.1 | Brief overview of overall study design

Bioinformatic analysis was conducted to acquire differentially expressed lncRNAs and messenger RNAs (mRNAs) as well as significantly enriched pathways and lncRNA/miRNA/mRNA axis in AKI. Dual-luciferase reporter gene assay was conducted to verify the targeted relationship between miRNA and lncRNA as well as miRNA and mRNA. Establishment of I/R cell model and renal I/R injury experiment in rats were carried out to verify the roles of LINC00520 in AKI progression in vitro and in vivo, respectively. Measurement of reactive oxygen specie (ROS) production and the amount of malondialdehyde (MDA) and superoxide dismutase (SOD) and PAS staining was carried out for detection of I/R injury in vitro and in vivo respectively. Besides, a figure regarding the overall study design is shown in the Supporting Information Data.

2.2 | Bioinformatic analysis

RNA expression data were downloaded from the NCBI Gene Expression Omnibus (GEO, GSE30718, https://www.ncbi.nlm.nih.gov/geo/). R software was used to perform differential analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Limma and heatmap packages were used for differential analysis. The Joy plot package, Rankplot package, and dot plot package in R studio (https://www.rstudio.com/) and Gene Set Enrichment Analysis (GSEA, http://software.broadinstitute.org/gsea/msigdb/index.jsp) running under the Java environment were used to conduct KEGG pathway enrichment analysis of differentially expressed genes (DEGs). A coexpression network among lncRNA, miRNA, and mRNA was constructed by Cytoscape software (https://cytoscape.org/).

2.3 | Cell culture

Human renal tubular epithelial cells (HK-2) were obtained from BeNa Culture Collection (Beijing, China) and cultured (37°C, 5% CO_2) in Dulbecco's modified Eagle's medium (DMEM; GibCo BRL, Grand Island, NY) with 10% fetal bovine serum (GibCo BRL), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO).

2.4 | Establishment of I/R cell model

HK-2 cells were incubated in six-well plates with glucose-free DMEM and then exposed to hypoxia in a humidified N_2 flushed hypoxic chamber for 4 hr. The cells were then maintained in complete DMEM and 21% O_2 for reoxygenation. No I/R cells were maintained in DMEM in the incubator under normoxic conditions (95% air/5% CO_2).

2.5 | Plasmid construction and dual-luciferase reporter gene assay

The wild- or mutation-type sequences of the candidate genes 3′-untranslated region (3′-UTR) targeting sites were inserted in the psiCHECK-2 luciferase reporter vector (C8021; Promega, Madison, WI) following the provider's instruction. Relative luciferase activity was measured by a microplate reader (Molecular Devices, Sunnyvale, CA).

2.6 | Cell transfection

The siRNA targeting LINC00520 (si-LINC00520), negative control (si-NC), and another transfection sequence like miR-27b-3p inhibitor, miR-27b-3p mimics were synthesized by Sangon Biotech (Shanghai, China). OSM receptor β (*OSMR*) encodes a member of the type I cytokine receptor family, and *OSMR* sequence was cloned into pLVX vector by Sangon Biotech (Shanghai, China). For in vitro transfection, HK-2 cells were transfected using 4 μl Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

2.7 | Western blot

Total proteins were extracted from HK-2 cells using RIPA lysis buffer (Takara Biotechnology, Dalian, China). A total of 20 μg proteins were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and blotted on polyvinylidene difluoride membranes (Millipore Corp., Billerica, MA). After blocking in Tris-buffered saline (TBS) buffer (50 mmol/L NaCl, 10 mmol/L Tris, pH 7.4) containing 5% nonfat milk, membranes were reacted with primary antibodies at 4°C overnight. The primary antibodies were anti-Bcl-2, anti-Bax, anti-p-PI3K, anti-*OSMR*, anti-p-Akt, and antiglyceraldehyde 3-phosphate dehydrogenase (GAPDH; Abcam, Shanghai, China). Subsequently, the membranes were incubated with antimouse IgG secondary antibodies (Abcam) at room temperature for 1 hr. Finally, the immunoreactive protein bands were visualized using enhanced chemiluminescence reagents (Amersham, Little Chalfont, UK).

2.8 | Flow cytometry

HK-2 cells were seeded in six-well plates at a density of 1×10^5 cells/well. After treatment, the cells were trypsinized and incubated in 500 μl of binding buffer containing 5 μl of Annexin V-FITC and 5 μl of propidium iodide for 30 min in the dark. Afterward, the cells were analyzed with a flow cytometer (Invitrogen).

TABLE 1 Sequences of primers

Genes	Sequence (5′–3′)
OSMR	Forward: ATGGCTCTATTTGCAGTCTTTCA Reverse: CACCCAGATGACATTGGATGTT
LINC00520	Forward: GGGAGTAAGAGGTGTGGCAA Reverse: CCATGGCCATTTTGCAAGGA
miR-27b-3p	Forward: ACACTCCAGCTGGGTTTCACAGTGGCTAAG Reverse: TGGTGTCGTGGAGTCG
U6	Forward: GCGCGTCGTGAAGCGTTC Reverse: GTGCAGGGTCCGAGGT
GAPDH	Forward: GGAGCGAGATCCCTCCAAAAT Reverse: GGCTGTTGTCATACTTCTCATGG

Note. GADPH: glyceraldehyde 3-phosphate dehydrogenase; OSMR: oncostatin M receptor β.

2.9 | Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from HK-2 cells using TRIzol reagent (Invitrogen). The first-strand cDNA was synthesized from 1 μg of total RNA using Prime Script RT reagent Kit with gDNA Eraser (TaKaRa, Shimogyo-ku, Kyoto, Japan). We analyzed the relative quantity of mRNA using the $2^{-\Delta\Delta C_t}$ methods and the internal control to U6 and GAPDH. The primer sequences used in qRT/PCR are shown in Table 1.

2.10 | Animal care

Adult male Sprague-Dawley (SD) rats (300–350 g) were used in animal experiments in accordance with the instructions of the Department of Neurology Intensive Care Unit, The Affiliated Yantai Yuhuangding Hospital of Qingdao University. All experimental animal procedures were approved by the Institutional Animal Care and Use Committee of The Affiliated Yantai Yuhuangding Hospital of Qingdao University. The rats were fed under the conditions of 22 ± 0.5°C, relative humidity 40–60%, and a standard 12-hr light-dark cycle.

2.11 | Renal I/R injury experiment

As for the renal I/R injury experiments, the rats were randomly and equally divided into I/R (n = 12) and I/R (n = 24). I/R group included si-NC group and si-LINC00520 group (n = 12 in each group). Each rat was anesthetized with intraperitoneal injection of 40 mg/kg sodium pentobarbital and kept a constant temperature using a heating pad. Renal I/R injury experiments for si-NC and si-LINC005020 group were conducted referring to accepted protocols as follows. In brief, bilateral renal nephrons were exposed after the abdominal cavity was incised in the midline. Renal I/R injury was induced without trauma by clamping both renal pedicles for 1 hr. When the kidney color turned from dark to pink, the clamps were removed and the blood flow was restored. Before the abdominal cavity was sutured, 1 ml warm normal saline was injected to reduce internal air. In the no I/R group, 12 adult male SD rats were injected with normal saline

into the subcutaneous tissue of the right flank as a control. In the I/R group, 12 adult male SD rats were injected with HK-2 cells transfected with si-LINC into the subcutaneous tissue of the right flank, and 12 adult male SD rats were injected with HK-2 cells transfected with si-NC. Rats were suffocated to death using CO_2 24 hr after experiments. Kidneys specimens were conserved at −80°C for further use.

2.12 | I/R injury detection

Intracellular ROS production was detected using the fluorescence probe 2′,7′-dichlorodihydrofluorescein diacetate (DCHF-DA; Jiancheng Biotech, Nanjing, China). Briefly, after treatment, HK-2 cells were rinsed twice with PBS and then stained with 10 μM DCFH-DA for 30 min at 37°C in the dark. Fluorescence of DCFH-DA was measured with a fluorescence microscope at 485 nm excitation and 535 nm. After treatment, the cell culture medium was centrifuged, and the supernatant was collected. The levels of MDA and SOD in the supernatant were measured using Lipid Peroxidation MDA Assay Kit and Total SOD Assay Kit with WST-8 (Beyotime, Jiangsu, China) according to the manufacturer's instruction. The values of different MDA and SOD activities were expressed as a percentage of the control, respectively.

2.13 | Periodic acid–Schiff (PAS) staining

PAS staining was used for detection of I/R injury in rats in vivo. For PAS staining, kidney specimens were first fixed with 10% formalin solution and embedded with paraffin. Then, 3-μm-thick renal tissue sections were cut and stained with periodic PAS. The kidney injuries were scored according to the rules made by (Q. Q. Liu et al., 2017.

2.14 | Terminal deoxynucleotidyl Transfer-Mediated dUTP nick End-Labeling (TUNEL) staining

Cryostat sections of the kidney were fixed in 100% ethanol for 10 min at room temperature and then allowed to air dry completely. For TUNEL staining, sections were incubated with terminal deoxynucleotidyl transferase buffer (30 mmol/L Tris, 140 mmol/L sodium cacodylate, 1 mmol/L cobalt chloride, pH 7.2) containing terminal deoxynucleotidyl transferase (0.5 U/mL) and fluorescein-12-dUTP (2′-deoxyuridine 5′-triphosphate; 0.04 mmol/L; all reagents from Boehringer-Mannheim, Indianapolis, IN) in a total volume of 30 μl/slide, for 1 hr at 37°C. The TUNEL reaction was terminated by immersing the slide in 100% ethanol, and slides were coverslipped in Permount (Fisher Scientific, NH).

2.15 | Statistical analysis

Statistical analysis was carried out by the GraphPad Prism 6.0 software and data was presented as mean ± SD in this study. Statistical analysis was carried out using Student t-test and two-way analysis of variance (ANOVA) combined with Bonferroni test. $P < 0.05$ was considered significant.

2.16 | Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Affiliated Yantai Yuhuangding Hospital of Qingdao University.

3 | RESULTS

3.1 | LINC00520 was upregulated and PI3K/Akt pathway was activated in AKI

Microarray analysis was used to screen out differentially expressed mRNAs and lncRNAs to explore the molecular mechanism of kidney transplants acute injury. Figure 1a indicates top 10 upregulated and downregulated mRNAs in AKI. Besides, the results of the differential analysis showed that only CTSLP8 and LINC00520 were highly expressed in AKI (Figure 1b). KEGG pathway analysis showed the activated and inhibited pathways in AKI (Figure 1c). In the peak map of pathway enrichment analysis, all peaks of pathway were on the right side of zero, which indicated that all pathways were activated (Figure 2a). In a bubble map of pathway enrichment analysis, PI3k/Akt was listed in red group, which also meant it was activated (Figure 2b). In Figure 2c, every dark line was on behalf of a gene. From that, most of genes were enriched above the zero, indicating that the PI3K/Akt pathway was activated. Thereafter, the expression data of genes that were not only dysregulated in AKI tissues but also involved in PI3K-AKT signaling pathway are shown in Figure 2d. Among them, *OSMR* was one of overexpressed genes in AKI.

3.2 | LINC00520/miR-27b-3p/*OSMR* axis in AKI

With the use of Cytoscape, we produced a coexpression network to study the lncRNAs and mRNAs as well as the relationship among them clearly shown in Figure 3a. The results showed that LNC00520 was coexpressed with *OSMR*, and both were upregulated in AKI. Moreover, we filtrated 14 miRNAs that could target both *OSMR* and LINC00520 (Figure 3b). As miR-27b-3p was few studied in AKI and miR-27b-3p was closely related to inflammatory response and tumor cell malignant proliferation, we selected miR-27b-3p as our research object. There are potential binding sites at the 3′- and 5′-UTR among the selected LINC00520-miR-27b-3p-*OSMR* (Figure 3c). Furthermore, qRT-PCR was performed to verify the expression of LINC00520, miR-27b-3p, and *OSMR* in normal and AKI renal tissues. The results suggested that LINC00520 and *OSMR* were observably upregulated in AKI renal tissues, while miR-27b-3p was markedly downregulated in AKI renal tissues (Figure 3d). Besides, dual luciferase reporter gene assays indicated that LINC00520-WT and *OSMR*-WT presented lower luciferase activity than the corresponding MUT group, confirming the binding relationship of LINC00520/miR-27b and miR-27b-3p/*OSMR* (Figure 3e,f).

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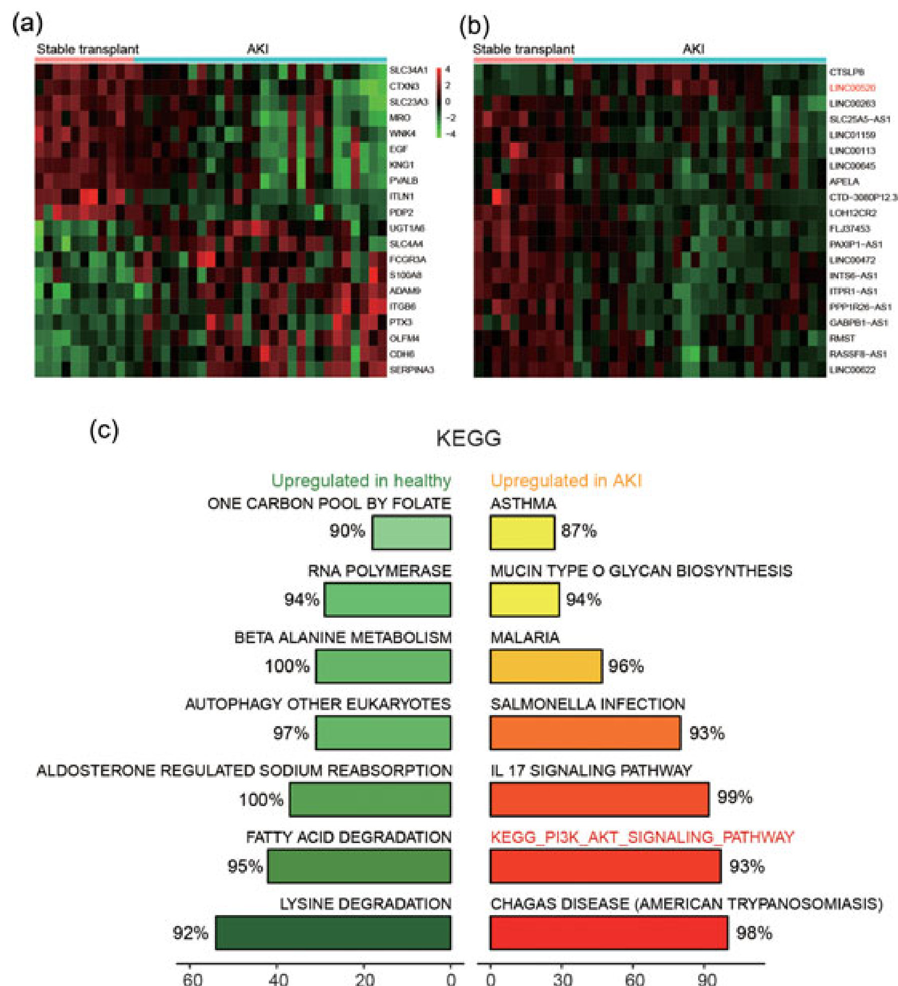

FIGURE 1 Differential mRNAs and lncRNAs expressions analysis in AKI. (a) Differential analysis heatmap of mRNAs in stable transplant and AKI mice. (b) Different lncRNAs expression in stable transplant and AKI mice. (c) KEGG Pathway Enrichment Analysis by Rank plot [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | miR-27b-3p inhibitor restrained suppression si-LINC00520 induced on renal I/R injury in vitro

To verify the efficiency of knockdown LINC00520 and miR-27b-3p in HK-2 cells, qRT-PCR was performed. The results showed that LINC00520 was significantly reduced in si-LINC cells and miR-27b-3p inhibitor (miR-inhibitor) also significantly reduced miR-27b-3p expression compared with the miR-NC group (Figure 4a,b). In contrast with no I/R HK-2 cells, the ROS level of I/R HK-2 cells group was obviously upregulated. After knockdown of LINC00520, I/R-induced ROS levels were decreased. The addition of miR-27b-3p inhibitors contributed to the increase of ROS level to some extent (Figure 4c). MDA levels showed a trend similar to ROS, while SOD performed a reverse trend (Figure 4d,e). The flow cytometry experiment indicated that the apoptosis rate of I/R HK-2 cells was obviously enhanced and downregulated LINC00520 expression further reversed the apoptosis, and the apoptosis rate rose again after adding miR-27b-3p inhibitor (Figure 4f,g). The results of western blot showed that knockdown of LINC00520 significantly reduced the high expression of apoptosis protein Bax induced by I/R injury, and transfection of miR-27b-3p inhibitor increased Bax again. However, the trend of antiapoptosis protein Bcl-2 was opposite to that of Bax (Figure 4h,i).

3.4 | *OSMR* impaired the Recovery of Kidney Injury by miR-27b-3p

Transfection efficiency experiments showed that both miR-27b-3p and *OSMR* expression levels in HK-2 cells were increased significantly after transfection with miR-27b-3p mimics and *OSMR* (Figure 5a,b). miR-27b-3p mimics caused a significant decrease to I/R-induced ROS levels. In contrast, *OSMR* overexpression increased ROS levels, and the addition of miR-27b-3p mimics contributed to the decrease of ROS level to some extent suggesting that miR-27b-3p mimics could reverse the action of *OSMR* (Figure 5c). MDA had the same trend similar to ROS, while the SOD level showed the opposite trend (Figure 5d,e). miR-27b-3p mimics reduced I/R-induced apoptosis, and the apoptosis rate of *OSMR* overexpression cells was increased (Figure 5f,g). Compared with the I/R-treated NC group, the level of Bax in the mimics group decreased, and increased after adding *OSMR*. The trend of Bcl-2 was opposite to that of Bax (Figure 5h,i).

3.5 | LINC00520 activated PI3k/Akt pathway to aggravate renal I/R injury in vitro

Compared with the no I/R-treated group, phosphorylation of PI3K and Akt in I/R HK-2 cells was obviously upregulated. After overexpression

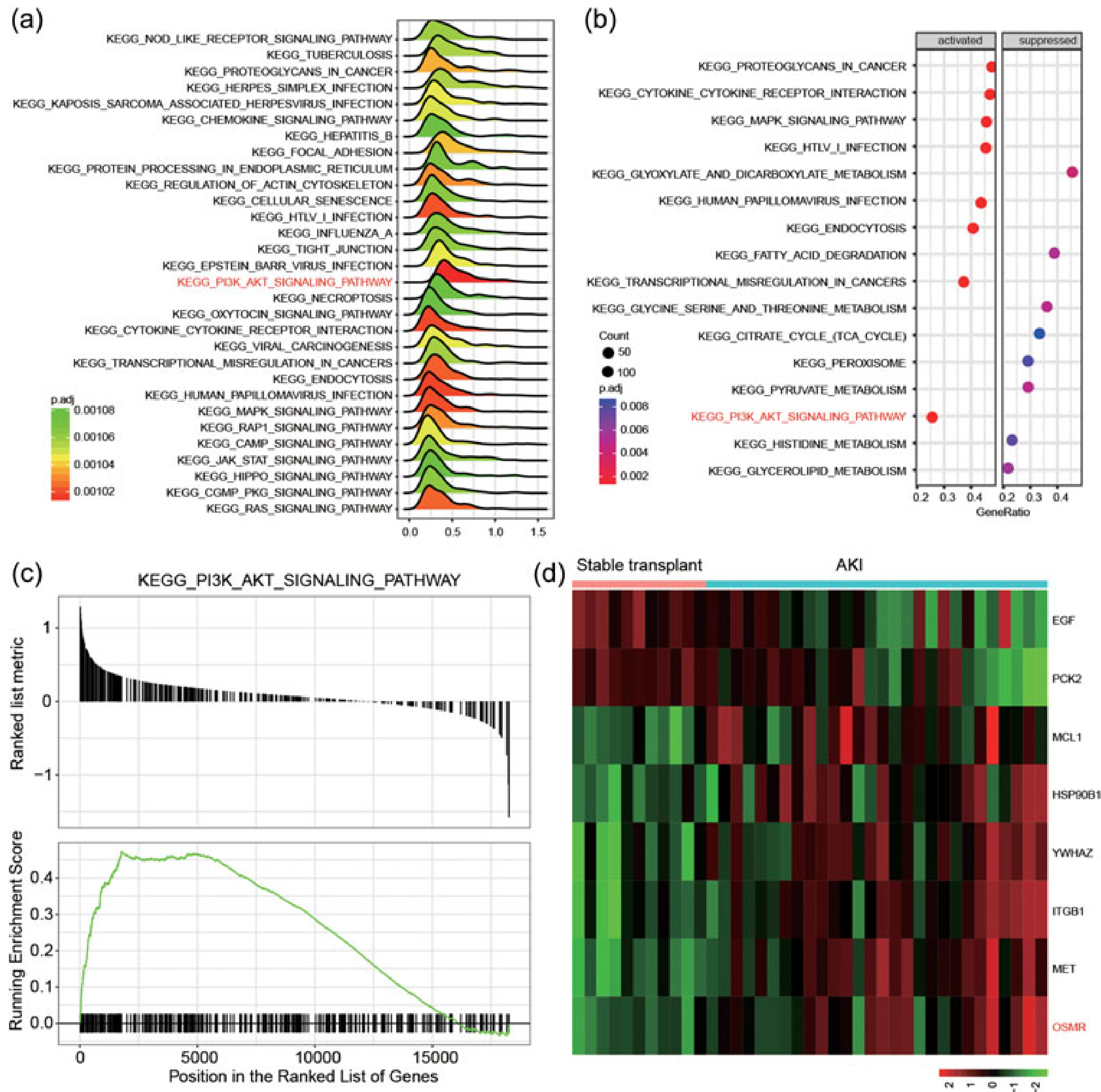

FIGURE 2 PI3K/Akt signaling pathway and its enrichment analysis. (a) PI3K/Akt pathway was upregulated in the mountain graph. (b) KEGG Pathway Enrichment Analysis by Joy plot. (c) KEGG Pathway Enrichment Analysis by dot plot. (d) The expression of PI3K/Akt signaling pathway related genes in stable transplant and AKI mice. OSMR was overexpressed in AKI according to the difference analysis heatmap [Color figure can be viewed at wileyonlinelibrary.com]

of LINC00520 in I/R HK-2 cells, phosphorylation of PI3K and Akt was further elevated (Figure 6a,b). To further verify the effect of LINC00520 on the PI3k/Akt signaling pathway, wortmannin, a PI3k/Akt signaling pathway inhibitor was used. As shown in Figure 6c–e, wortmannin reversed the effect of LINC00520 on ROS, MDA and SOD expression in I/R HK-2 cells. Compared with pLVX-NC, the apoptosis rate of LINC00520 group increased. After adding wortmannin, LINC00520-induced apoptosis rate decreased (Figure 6f,g). Compared with the pLVX-NC group, Bax in the LINC00520 group increased and then decreased after the addition of wortmanin execution, while the Bcl-2 trend was opposite to that of Bax (Figure 6h,i).

3.6 | Knockdown of LINC00520 reduced renal injury in rats

The PAS results of rat renal epithelial tissue sections showed that I/R treatment induced the atrophy of vascular glands compared with the sham group (Figure 7a). After knocking out the LINC00520, the atrophy of vascular glands was remission (Figure 7a). Tunel assay also indicated that I/R treatment enhanced the number of tunel positive cells and downregulation of LINC00520 expression significantly reduced tunel positive and inhibited apoptosis induced by I/R in vivo (Figure 7b). The qRT/PCR results demonstrated that the expression of miR-27b-3p was decreased after I/R treatment, and the expression

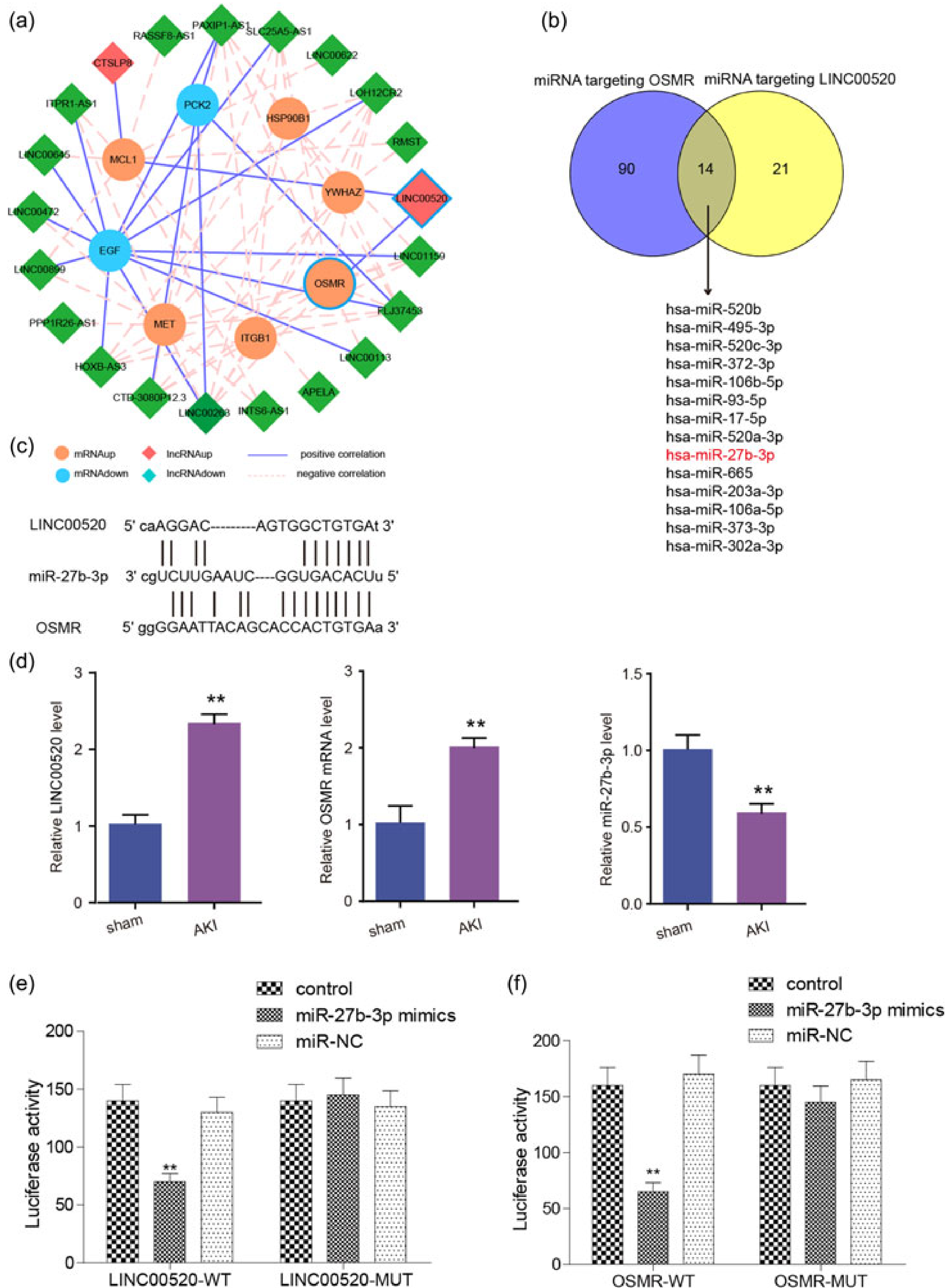

FIGURE 3 LINC00520/miR-27b-3p/*OSMR* axis implicated in AKI. (a) Cytoscape analysis results of lncRNA and mRNA. (b) Intersection of miRNAs which were predicted to target LINC00520 and *OSMR* both. (c) Potential binding sites of LINC00520, *OSMR*, and miR-27b-3p. (d) LINC00520, miR-27b-3p and *OSMR* expression in sham and AKI mice. $^{**}P < 0.01$, compared with sham group. (e,f) The dual luciferase reporter assays verified the binding relationship between LINC00520/miR-27b-3p and miR-27b-3p/*OSMR*. $^{**}P < 0.01$, compared miR-NC group [Color figure can be viewed at wileyonlinelibrary.com]

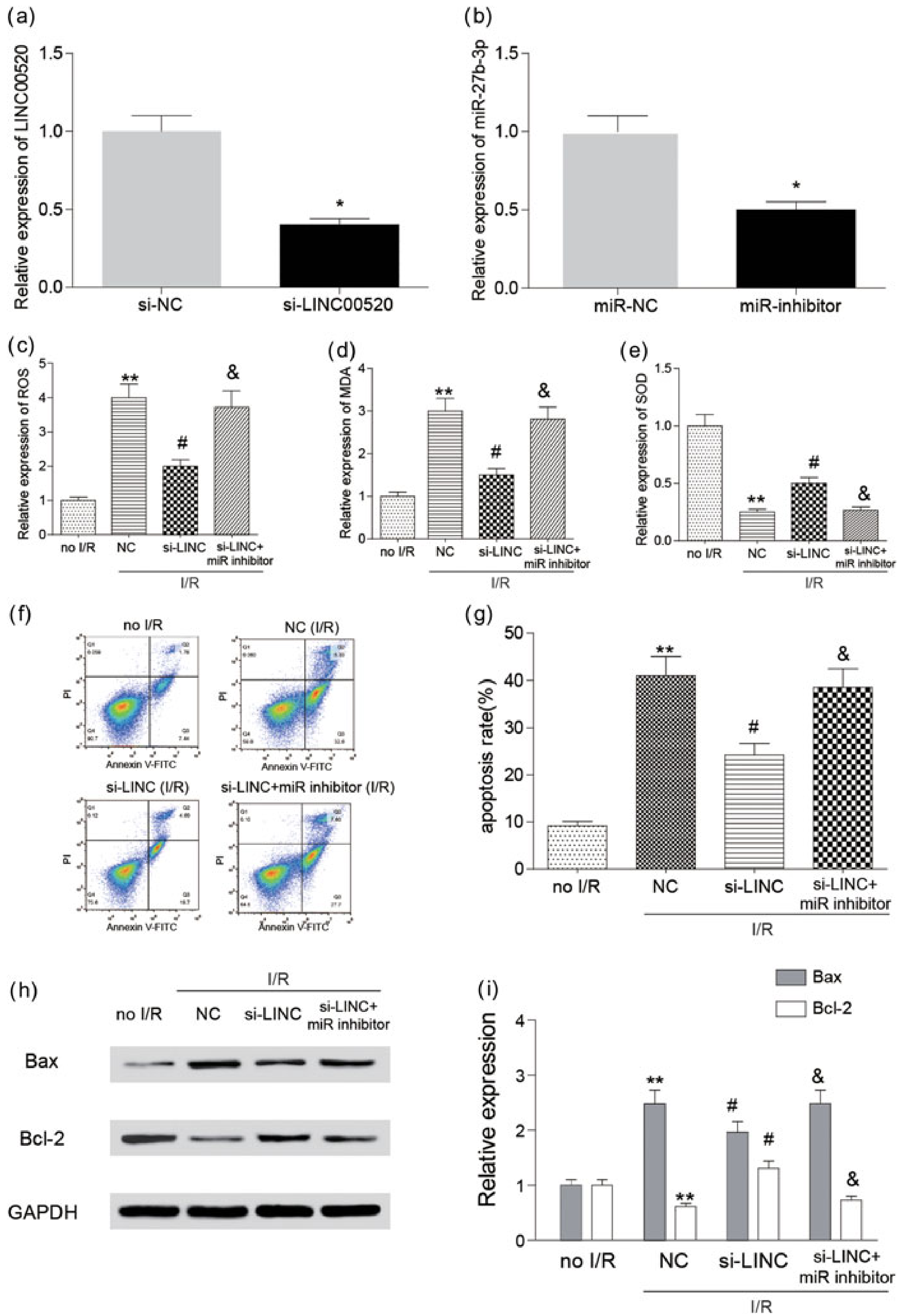

FIGURE 4 The impacts of LINC00520 and miR-27b-3p on I/R HK-2 cells. (a,b) Cell transfection efficiency of si-LINC and miR-27b-3p inhibitor. (c–e) The levels of ROS, MDA, and SOD under different conditions. (f,g) The results graph of flow cytometry experiments. (h,i) Expression of apoptosis-related proteins Bax and Bcl-2 by western blot. *$P < 0.05$, **$P < 0.01$ compared with no I/R group; $^{\#}P < 0.05$ compared with NC group; $^{\&}P < 0.05$ compared with si-LINC group [Color figure can be viewed at wileyonlinelibrary.com]

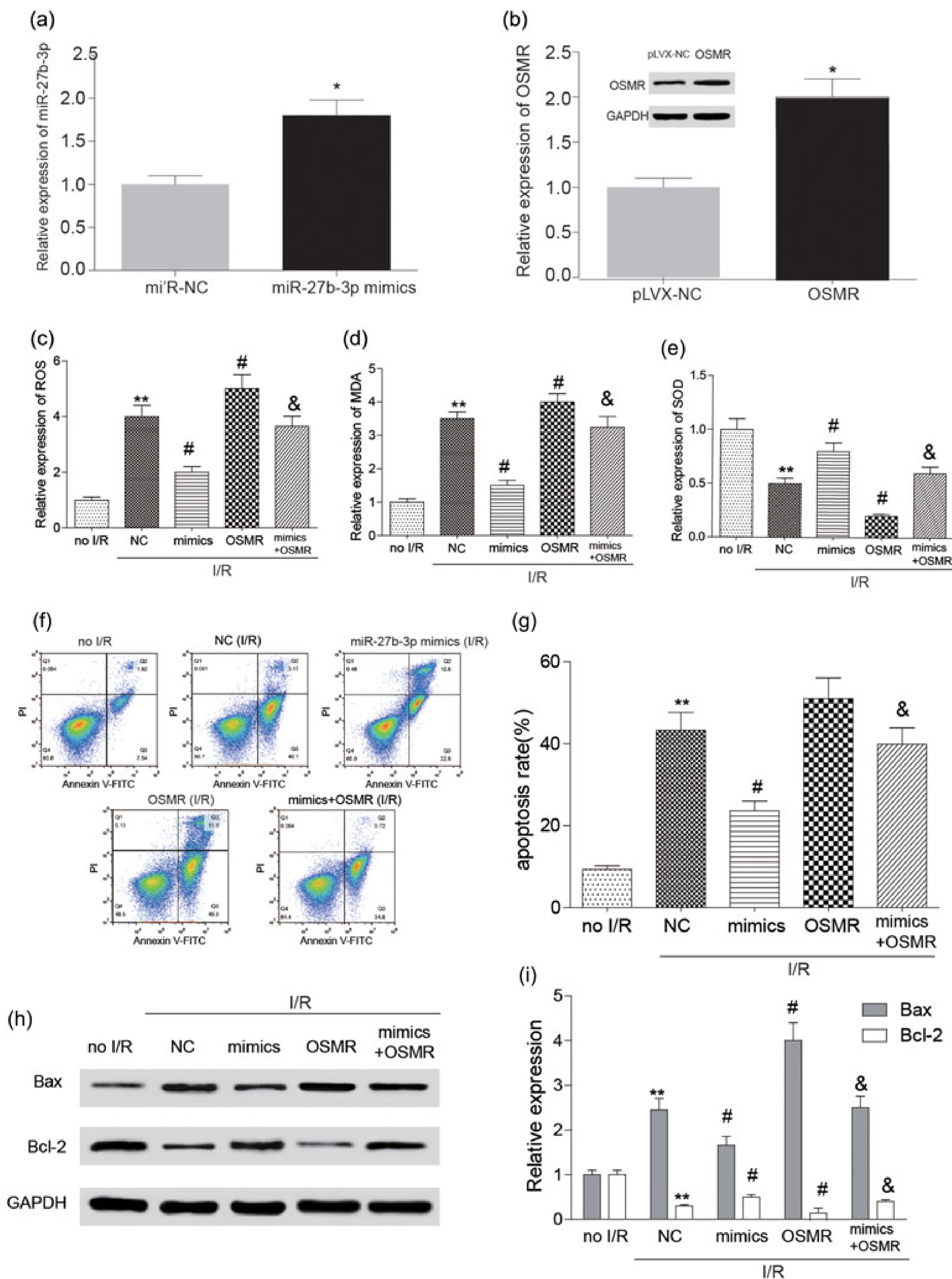

FIGURE 5 The impacts of miR-27b-3p and *OSMR* in I/R HK-2 cells. (a,b) Cell transfection efficiency of miR-27b-3p and *OSMR* overexpression. (c–e) Expression Levels of ROS, MDA, and SOD under different conditions. (f,g) The results graph of flow cytometry experiments. (h,i) Expression of apoptosis-related proteins Bax and Bcl-2 by western blot. *$P < 0.05$, **$P < 0.01$ compared with no I/R group; $^{\#}P < 0.05$ compared with NC group; $^{\&}P < 0.05$ compared with si-LINC group [Color figure can be viewed at wileyonlinelibrary.com]

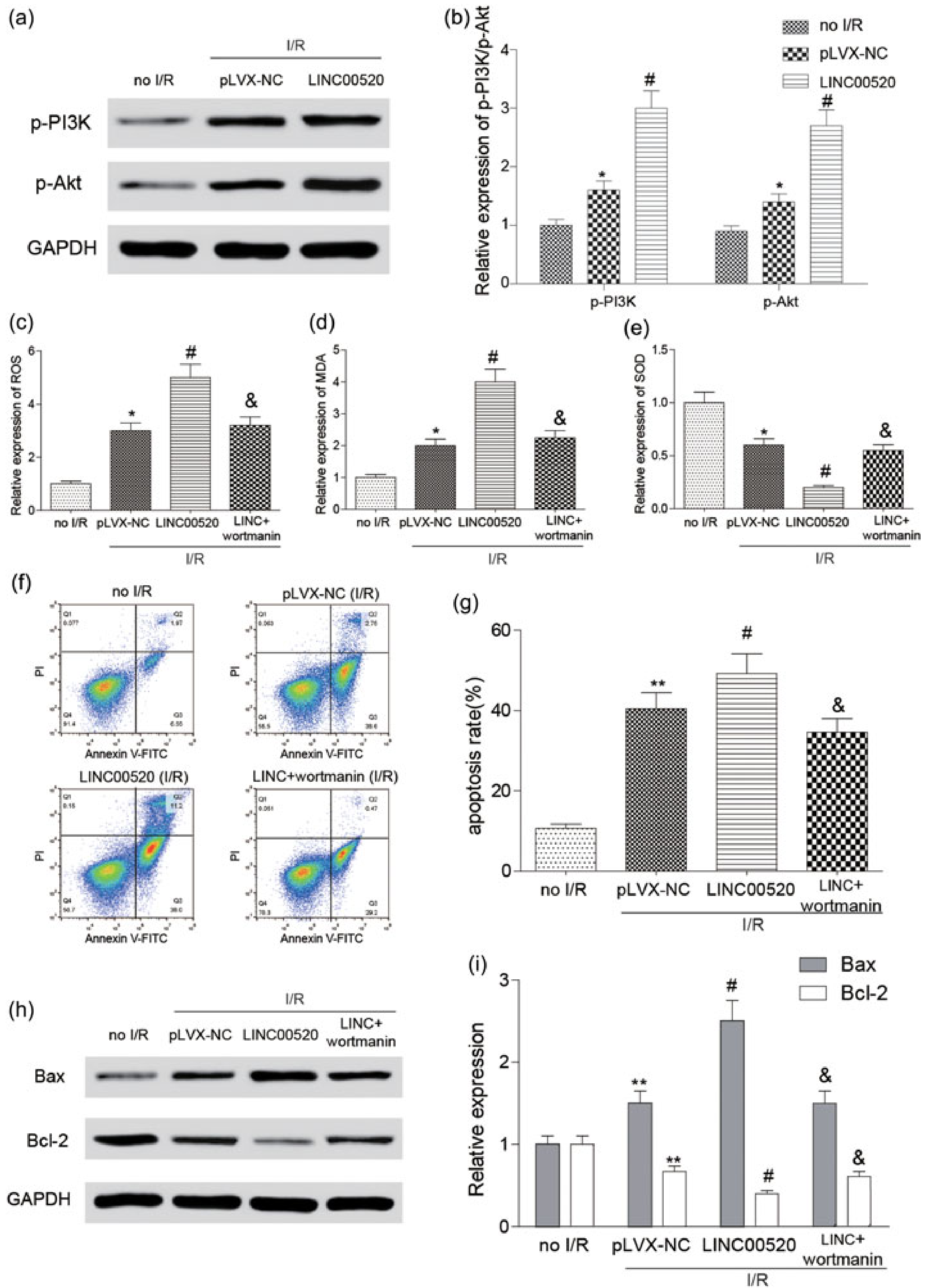

FIGURE 6 The relationship between LINC00520 and PI3K/Akt pathways in I/R HK-2 cells. (a,b) Expression levels of p-PI3K and p-Akt under different conditions. (c–e) Expression Levels of ROS, MDA, and SOD under different conditions. (f,g) The results graph of flow cytometry experiments. (h,i) Expression of apoptosis-related proteins Bax and Bcl-2 by western blot. $^*P < 0.05$, $^{**}P < 0.01$ compared with no I/R group; $^{\#}P < 0.05$ compared with NC group; $^{\&}P < 0.05$ compared with si-LINC group [Color figure can be viewed at wileyonlinelibrary.com]

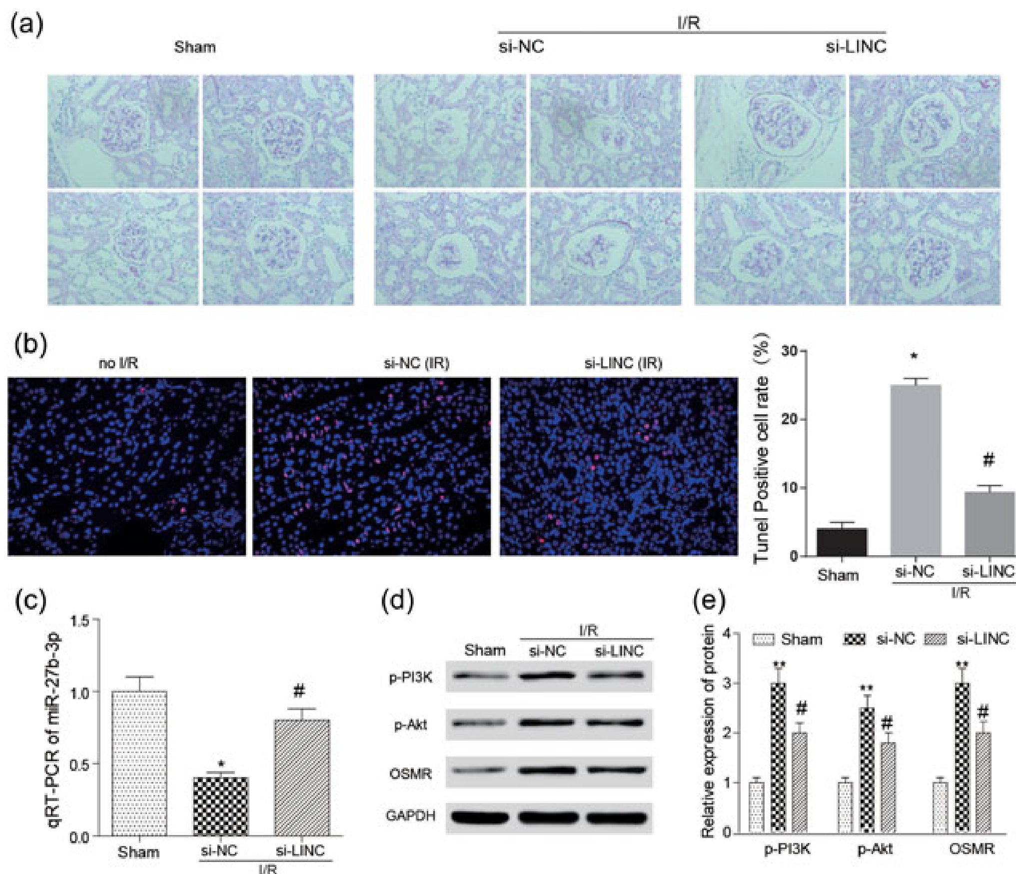

FIGURE 7 Effect of si-LINC00520 on rat renal I/R Injury in vivo. (a) PAS staining result of different groups. (b) Tunel staining of different groups. (c) Expression level of miR-27b-3p by qRT/PCR. (d,e) Western blot results of PI3K/Akt/OSMR in rat renal tissues. *$P < 0.05$, **$P < 0.01$, compared with sham group; ##$P < 0.05$ compared with si-NC group [Color figure can be viewed at wileyonlinelibrary.com]

of miR-27b-3p increased after knockdown of LINC00520 in vivo (Figure 7c). The Western blot results showed that the expression levels of *OSMR*, p-PI3k, and p-Akt in the I/R group were higher than in the sham group. After knockdown of the LINC00520, the levels of *OSMR*, p-PI3k, and p-Akt were decreased in turn (Figure 7d,e).

4 | DISCUSSION

In the current study, we analyzed the gene chip from GEO. Combined with the differential analysis and GSEA result, we found that LINC00520 and *OSMR* were both upregulated in the AKI group and the PI3K/AKT signaling pathway was activated in the AKI group from the joy plot and dot plot diagram. The pathway-specific gene analysis indicated that *OSMR* was also found to be significantly upregulated in the AKI group. The diagram from Cytoscape illustrated that the LINC00520 was coexpressed with *OSMR*, which was both upregulated in AKI. Besides, has-miR-27b-3p might be the regulator of LINC00520 and *OSMR*. To validate this hypothesis, a series of cell assays were performed. Luciferases assay validated that miR-27b-3p could target LINC00520 or *OSMR* respectively. Overexpression of LINC00520 promoted cell apoptosis rate of renal cells. Furthermore, we performed in vivo assay to validate the results. A semi-quantitative score by paller indicated that the si-LINC group was significantly different from the blank group, which suggested that LINC00520 might promote the progression of AKI. qRT-PCR suggested that miR-27b-3p was upregulated in the si-LINC group compared with the NC group, and western blot results indicated that the *OSMR* was downregulated while the expression of p-AKT and p-PI3K were also downregulated. All the results mentioned above suggested that LINC00520 could promote the progression of AKI by targeting miR-27b-3p/*OSMR* through the PI3K/AKT signaling pathway.

lncRNAs are emerging as important regulators of cellular function. Nevertheless, their specific role in renal disease is still not clear and need to be studies deeper. Lin et al. (2015) suggested that lnc-CPN2-1 might induce by hypoxia in renal, which was a kind of pathology of AKI, and they also gave a landscape of lncRNAs in both AKI and CKD. Huang et al. (2017) reported that PVT1 promoted the AKI by regulating TNF-α and JNK/NK-κB pathway. All research mentioned before indicated that lncRNA played an important role in the progression of AKI in our research, and we found that LINC00520 could promote the progression of AKI through the PI3K/AKT signaling pathway.

In a recent study, LINC00520 has been proven to be upregulated in tumor tissues and they also verified that LINC00520 is regulated by the PI3K/AKT signaling pathway in breast cancer (Henry et al., 2016). The PI3K/AKT signaling pathway has been studied a lot in renal diseases. Recent research suggests that salvianolic acid B can protect against AKI

by suppression of oxidative stress through the PI3K/AKT pathway (Tongqiang et al., 2016). Another study by Fu et al. (2017) indicated that miR-21 could protect the renal cell by targeting PTEN through the PI3K/AKT pathway. In our research, LINC00520 was found out to promote the process of AKI through the PI3K/AKT signaling pathway, which was partly consistent with the previous research and this study is the first to combine lncRNA, miRNA, and mRNA with the signaling pathway. LINC00520 has been proved to target miR-27b-3p to regulate the expression level of *OSMR* through the PI3K/AKT signaling pathway, which was a novel aspect for the therapeutic direction for AKI.

As another noncoding RNA, miRNA, the functions of miRNAs in AKI progression have been studied a lot in previous studies. miR-21 in urine and plasma were proved to be associated with severe AKI (Du et al., 2013). Several specific miRNAs, such as miR-687 (Bhatt et al., 2015), miR-489 (Wei et al., 2016), miR-494 (Lan et al., 2012), miR-24 (Lorenzen et al., 2014), and miR-126 (Bijkerk et al., 2014), were also been investigated in subsequent studies. The miRNA we studied in our research was miR-27b-3p, which was widely studied in lung cancer, hepatocellular carcinoma, and breast cancer. Previous research pointed out that the lower expression of miR-27b-3p might affect the triple negative breast cancer (Liu et al., 2015). Another study on breast cancer also suggested that miR-27b-3p is an antitumor gene, and the expression level was low in the tumor tissue (Sun, Xu, Cao, & Ding, 2017). The expression of miR-27b-3p is also low in our research, which matched with the previous studies.

OSMR encodes a member of the type I cytokine receptor family. Recent studies pointed out that the mutation on *OSMR* might arouse familial primary localized cutaneous amyloidosis (Wali et al., 2015). Another research reported that renal parenchyma is capable of generating a strong acute phase response, likely mediated via OSM/*OSMR* (Luyckx et al., 2009). In our study, *OSMR* was regulated by LINC00520 to promote the progression of AKI via PI3K/AKT signaling pathway, which is a novel research thoughts for the pathology of AKI.

The relation between miR-27b-3p and LINC00520 was validated in our research by luciferase assay and the results suggested that LINC00520 can target to miR-27b-3p. lncRNA has been found that they can serve as the ceRNA which was newly discovered in recent years (Xie et al., 2018). In our study, LINC00520 acted as a ceRNA to competitively inhibit the miR-27b-3p in AKI progression and this lncRNA-miRNA regulation might also affect the expression of *OSMR*, which as verified in our experiments. The specific route of this regulation was conducted through the PI3K/AKT signaling pathway. Our results indicated that LINC00520 could competitively inhibit the miR-27b-3p through the PI3K/AKT pathway. *OSMR* was upregulated in AKI by miR-27b-3p through the PI3K/AKT pathway, and all the results indicated that could promote the progression of AKI.

The limitation of our study is that we only used one cell line to validate our hypothesis and more cell lines will be studied in the future. Another limitation of our study is that other miRNAs that can be regulated by LINC00520 might be studied in the further study. Our assay has proved a clear molecular mechanism of AKI process, which provides a novel therapeutic method for AKI.

In conclusion, we have proved that LINC00520 can target miR-27b-3p to regulate the expression level of *OSMR* through the PI3K/AKT signaling pathway to promote the progression of AKI.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

Bhatt, K., Wei, Q., Pabla, N., Dong, G., Mi, Q. S., Liang, M., … Dong, Z. (2015). MicroRNA-687 induced by hypoxia-inducible factor-1 targets phosphatase and tensin homolog in renal ischemia-reperfusion injury. *Journal of the American Society of Nephrology*, *26*(7), 1588–1596.

Bijkerk, R., van Solingen, C., de Boer, H. C., van der Pol, P., Khairoun, M., de Bruin, R. G., … van Zonneveld, A. J. (2014). Hematopoietic microRNA-126 protects against renal ischemia/reperfusion injury by promoting vascular integrity. *Journal of the American Society of Nephrology*, *25*(8), 1710–1722.

Chang, J. W., Jeng, M. J., Yang, L. Y., Chen, T. J., Chiang, S. C., Soong, W. J., … Tsai, H. L. (2015). The epidemiology and prognostic factors of mortality in critically ill children with acute kidney injury in Taiwan. *Kidney International*, *87*(3), 632–639.

Chen, Z., Ding, H. S., Guo, X., Shen, J. J., Fan, D., Huang, Y., & Huang, C. X. (2018). miR-33 promotes myocardial fibrosis by inhibiting MMP16 and stimulating p38 MAPK signaling. *Oncotarget*, *9*(31), 22047–22057.

Coca, S. G., Singanamala, S., & Parikh, C. R. (2012). Chronic kidney disease after acute kidney injury: A systematic review and meta-analysis. *Kidney International*, *81*(5), 442–448.

Ding, J., Yeh, C. R., Sun, Y., Lin, C., Chou, J., Ou, Z., … Yeh, S. (2018). Estrogen receptor beta promotes renal cell carcinoma progression via regulating lncRNA HOTAIR-miR-138/200c/204/217 associated CeRNA network. *Oncogene*, *37*(37), 5037–5053.

Du, J., Cao, X., Zou, L., Chen, Y., Guo, J., Chen, Z., … Zheng, Z. (2013). MicroRNA-21 and risk of severe acute kidney injury and poor outcomes after adult cardiac surgery. *PLOS One*, *8*(5), e63390.

ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature*, *489*(7414), 57–74.

Fu, D., Dong, J., Li, P., Tang, C., Cheng, W., Xu, Z., … Zhang, Z. (2017). miRNA-21 has effects to protect kidney injury induced by sepsis. *Biomedicine and Pharmacotherapy*, *94*, 1138–1144.

He, P., Sun, L., Zhu, D., Zhang, H., Zhang, L., Guo, Y., … Xie, P. (2016). Knock-down of endogenous bornavirus-like nucleoprotein 1 inhibits cell growth and induces apoptosis in human oligodendroglia cells. *International Journal of Molecular Sciences*, *17*(4), 435.

He, X., Teng, J., Cui, C., Li, D., & Wen, L. (2018). MicroRNA-182 inhibits HCMV replication through activation of type I IFN response by targeting FOXO3 in neural cells. *Experimental Cell Research*, *369*(2), 197–207.

Henry, W. S., Hendrickson, D. G., Beca, F., Glass, B., Lindahl-Allen, M., He, L., … Toker, A. (2016). LINC00520 is induced by Src, STAT3, and PI3K and plays a functional role in breast cancer. *Oncotarget*, *7*(50), 81981–81994.

Huang, W., Lan, X., Li, X., Wang, D., Sun, Y., Wang, Q., … Yu, K. (2017). Long non-coding RNA PVT1 promote LPS-induced septic acute kidney injury by regulating TNFalpha and JNK/NF-kappaB pathways in HK-2 cells. *International Immunopharmacology*, *47*, 134–140.

Komori, T., Tanaka, M., Senba, E., Miyajima, A., & Morikawa, Y. (2013). Lack of oncostatin M receptor beta leads to adipose tissue inflammation and insulin resistance by switching macrophage phenotype. *Journal of Biological Chemistry*, *288*(30), 21861–21875.

Komori, T., Tanaka, M., Senba, E., Miyajima, A., & Morikawa, Y. (2014). Deficiency of oncostatin M receptor beta (*OSMR*beta) exacerbates high-fat-diet-induced obesity and related metabolic disorders in mice. *Journal of Biological Chemistry*, *289*(20), 13821–13837.

Lan, Y. F., Chen, H. H., Lai, P. F., Cheng, C. F., Huang, Y. T., Lee, Y. C., … Lin, H. (2012). MicroRNA-494 reduces ATF3 expression and promotes AKI. *Journal of the American Society of Nephrology*, *23*(12), 2012–2023.

Lin, J., Zhang, X., Xue, C., Zhang, H., Shashaty, M. G. S., Gosai, S. J., … Reilly, M. P. (2015). The long noncoding RNA landscape in hypoxic and inflammatory renal epithelial injury. *American Journal of Physiology. Renal Physiology*, *309*(11), F901–F913.

Liu, L., Xu, L., Zhang, S., Wang, D., Dong, G., Chen, H., … Wang, R. (2018). STF-083010, an inhibitor of XBP1 splicing, attenuates acute renal failure in rats by suppressing endoplasmic reticulum stress-induced apoptosis and inflammation. *Experimental Animals*, *67*(3), 373–382.

Liu, Q. Q., Liu, H., He, Z. G., Zhang, S. J., Liu, B. W., Wang, L., … Lv, Y. M. (2017). Differential gene and lncRNA expression in the lower thoracic spinal cord following ischemia/reperfusion-induced acute kidney injury in rats. *Oncotarget*, *8*(32), 53465–53481.

Liu, Y., Cai, Q., Bao, P. P., Su, Y., Cai, H., Wu, J., … Shu, X. O. (2015). Tumor tissue microRNA expression in association with triple-negative breast cancer outcomes. *Breast Cancer Research and Treatment*, *152*(1), 183–191.

Lorenzen, J. M., Kaucsar, T., Schauerte, C., Schmitt, R., Rong, S., Hubner, A., … Thum, T. (2014). MicroRNA-24 antagonism prevents renal ischemia-reperfusion injury. *Journal of the American Society of Nephrology*, *25*(12), 2717–2729.

Lorenzen, J. M., Schauerte, C., Kielstein, J. T., Hubner, A., Martino, F., Fiedler, J., … Thum, T. (2015). Circulating long noncoding RNATapSaki is a predictor of mortality in critically ill patients with acute kidney injury. *Clinical Chemistry*, *61*(1), 191–201.

Luyckx, V. A., Cairo, L. V., Compston, C. A., Phan, W. L., & Mueller, T. F. (2009). Oncostatin M pathway plays a major role in the renal acute phase response. *American Journal of Physiology. Renal Physiology*, *296*(4), F875–F883.

Lv, J., Kong, Y., Gao, Z., Liu, Y., Zhu, P., & Yu, Z. (2018). lncRNA TUG1 interacting with miR-144 contributes to proliferation, migration and tumorigenesis through activating the JAK2/STAT3 pathway in hepatocellular carcinoma. *International Journal of Biochemistry and Cell Biology*, *101*, 19–28.

Odutayo, A., Wong, C. X., Farkouh, M., Altman, D. G., Hopewell, S., Emdin, C. A., & Hunn, B. H. (2017). AKI and long-term risk for cardiovascular events and mortality. *Journal of the American Society of Nephrology*, *28*(1), 377–387.

Plesner, L., & Plesner, I. W. (1988). Distinction between the intermediates in Na+-ATPase and Na+, K+-ATPase reactions. I. Exchange and hydrolysis kinetics at millimolar nucleotide concentrations. *Biochimica et Biophysica Acta/General Subjects*, *937*(1), 51–62.

Pollard, J., Burns, P. A., Hughes, T. A., Ho-Yen, C., Jones, J. L., Mukherjee, G., … Shaaban, A. M. (2018). Differential expression of microRNAs in breast cancers from four different ethnicities. *Pathobiology*, *85*(4), 220–226.

Sun, Y., Xu, T., Cao, Y. W., & Ding, X. Q. (2017). Antitumor effect of miR-27b-3p on lung cancer cells via targeting Fzd7. *European Review for Medical and Pharmacological Sciences*, *21*(18), 4113–4123.

Tamer, L., Polat, G., Yucebilgic, G., Guvenc, B., & Baslamisli, F. (2000). The levels of sera malondialdehyde, erythrocyte membrane Na+-K+/Mg++ and Ca++/Mg++ adenosine 5′ triphosphatase in patients with sickle cell anemia. *Turkish Journal of Hematology*, *17*(1), 23–26.

Tang, P. M. K., Zhang, Y. Y., Mak, T. S. K., Tang, P. C. T., Huang, X. R., & Lan, H. Y. (2018). Transforming growth factor-beta signalling in renal fibrosis: From Smads to noncoding RNAs. *Journal of Physiology*, *596*(16), 3493–3503.

Tongqiang, L., Shaopeng, L., Xiaofang, Y., Nana, S., Xialian, X., Jiachang, H., … Xiaoqiang, D. (2016). Salvianolic acid B prevents iodinated contrast media-induced acute renal injury in rats via the PI3K/Akt/Nrf2 pathway. *Oxidative Medicine and Cellular Longevity*, *2016*, 7079487.

Vanmassenhove, J., Kielstein, J., Jörres, A., & Biesen, W. V. (2017). Management of patients at risk of acute kidney injury. *Lancet*, *389*(10084), 2139–2151.

Wali, A., Liu, L., Takeichi, T., Jelani, M., Rahman, O., Heng, Y., … Betz, R. (2015). Familial primary localized cutaneous amyloidosis results from either dominant or recessive mutations in OSMR. *Acta Dermato-Venereologica*, *95*(8), 1005–1007.

Wang, Y., Lu, Z., Wang, N., Feng, J., Zhang, J., Luan, L., … Zeng, X. (2018). Long noncoding RNA DANCR promotes colorectal cancer proliferation and metastasis via miR-577 sponging. *Experimental & Molecular Medicine*, *50*(5), 57.

Wei, Q., Liu, Y., Liu, P., Hao, J., Liang, M., Mi, Q., … Dong, Z. (2016). MicroRNA-489 Induction by Hypoxia-Inducible Factor-1 Protects against Ischemic Kidney Injury. *Journal of the American Society of Nephrology*, *27*(9), 2784–2796.

Winder, D. M., Chattopadhyay, A., Muralidhar, B., Bauer, J., English, W. R., Zhang, X., … Coleman, N. (2011). Overexpression of the oncostatin M receptor in cervical squamous cell carcinoma cells is associated with a pro-angiogenic phenotype and increased cell motility and invasiveness. *Journal of Pathology*, *225*(3), 448–462.

Xie, Q., Wu, G. Z., Yang, N., Shen, Y. H., Tang, J., & Zhang, W. D. (2018). Delavatine A, an unusual isoquinoline alkaloid exerts anti-inflammation on LPS-induced proinflammatory cytokines production by suppressing NF-kappaB activation in BV-2 microglia. *Biochemical and Biophysical Research Communications*, *502*(2), 202–208.

Xu, T., Wang, W., Ma, P., Shuai, Y., Zhao, K., Wang, Y., … Shu, Y. (2018). Upregulation of the long noncoding RNA FOXD2-AS1 promotes carcinogenesis by epigenetically silencing EphB3 through EZH2 and LSD1, and predicts poor prognosis in gastric cancer. *Oncogene*, *37*(36), 5020–5036.

Xu, X., Nie, S., Liu, Z., Chen, C., Xu, G., Zha, Y., … Hou, F. F. (2015). Epidemiology and clinical correlates of AKI in Chinese hospitalized adults. *Clinical Journal of the American Society of Nephrology*, *10*(9), 1510–1518.

Yu, T. M., Palanisamy, K., Sun, K. T., Day, Y. J., Shu, K. H., Wang, I. K., … Li, C. Y. (2016). RANTES mediates kidney ischemia reperfusion injury through a possible role of HIF-1alpha and lncRNA PRINS. *Scientific Reports*, *6*, 18424.

Zhou, P., Chen, Z., Zou, Y., & Wan, X. (2016). Roles of noncoding RNAs in acute kidney injury. *Kidney and Blood Pressure Research*, *41*(6), 757–769.

van Zonneveld, A. J., Rabelink, T. J., & Bijkerk, R. (2017). miRNA-coordinated networks as promising therapeutic targets for acute kidney injury. *American Journal of Pathology*, *187*(1), 20–24.

Zou, Y. F., & Zhang, W. (2018). Role of microRNA in the detection, progression, and intervention of acute kidney injury. *Experimental biology and medicine (Maywood, N.J.)*, *243*(2), 129–136.

Zuk, A., & Bonventre, J. V. (2016). Acute kidney injury. *Annual Review of Medicine*, *67*, 293–307.

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