European Review for Medical and Pharmacological Sciences 2018; 22: 8830-8838

MiR-122 regulates cell apoptosis and ROS by targeting DJ-1 in renal ischemic reperfusion injury rat models

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Abstract. – OBJECTIVE: Phosphatidylinositol 3-kinase/protein kinase B ((PI3K/AKT) signaling pathway plays a role in regulating cell survival and apoptosis. Phosphatase and tensin homologue deleted on chromosome ten (PTEN) can negatively regulate PI3K/AKT signaling pathway, while DJ-1 (Parkinson gene 7) can negatively regulate expression and function of PTEN. DJ-1-PTEN/PI3K/AKT signaling pathway plays a role in the regulation of ischemic reperfusion (IR) injury. Bioinformatics analysis showed that there was a targeted complementary binding site between microRNA-122 (miR-122) and 3′-UTR of DJ-1 mRNA. This study aimed to investigate the effects of miR-122 in regulating DJ-1-PTEN/PI3K/AKT signaling pathway [illegible] acute renal I-R injury.

MATERIALS AND METHODS: Rat renal artery was clamped and restored after 30 min to establish renal IR injury model. Renal tissue samples were collected at 10 h and 20 [illegible]eration. miR-122 and DJ-1 mRNA were detected with quantitative Real-time PCR (qRT-PCR). DJ-1 protein was tested by using Western blot. Blood urea nitrogen (BUN) and serum creatinine (SCr) were measured. Renal tubular epithelial cells, RRTEC, were cultured *in vitro* and divided into transfection (miR-NC [illegible] treatment group (miR-122 inhibitor group). IR treatment was performed after [illegible] h of transfection. DJ-1, PTEN, AKT, and phosphorylated AKT (p-AKT) were detected using Western blot. Cell apoptosis and reactive oxygen species (ROS) were measured with flow cytometry.

RESULTS: Compared with Sham group, blood BUN and SCr contents significantly increased ($p < 0.05$), miR-122 level significantly elevated ($p < 0.05$), while DJ-1 mRNA and protein markedly decreased ($p < 0.05$) in IR model rats. Compared with control group, I-R treatment significantly up-regulated miR-122 and PTEN expression ($p < 0.05$), decreased DJ-1 and p-AKT levels ($p < 0.05$), and induced apoptosis and ROS production ($p < 0.05$) in RRTEC cells. Transfection with miR-122 inhibitor markedly up-regulated DJ-1 expression ($p < 0.05$), enhanced PTEN/PI3K/AKT [illegible] activity ($p < 0.05$), and reduced [illegible] apoptosis [illegible] ROS production ($p < 0.05$).

CONCLUSIONS: MiR-122 increased significantly, while DJ-1 declined significantly during renal I-R injury. Down-regulation of miR-122 markedly elevated DJ-1, enhanced PTEN/PI3K/AKT pathway activity, and inhibited apoptosis and ROS generation in rat renal tubular epithelial cells to alleviate IR injury.

Key Words:

Kidney, Ischemia reperfusion, miR-122, DJ-1, Apoptosis, ROS.

Introduction

Ischemia reperfusion (IR) injury refers to the severe injury of tissues and organs caused by the ischemia and recovery of blood oxygen supply after a certain period of time. The functional damage of the target tissues and organs may even cause irreversible organic changes[1]. The kidney is rich in blood supply and highly perfused organ that is sensitive to ischemia and IR[2]. Phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway is widely expressed in many tissues and organs to regulate tissue development, cell proliferation and apoptosis[3]. The phosphatase and tensin homologue deleted on chromosome ten (PTEN) is a tumor suppressor gene to the transduction of PI3K/AKT signaling pathway. DJ-1 can increase the activity of PI3K/AKT signaling pathway by inhibiting the expression and function of PTEN to antagonize apoptosis, promote cell survival, and accelerate proliferation[4]. It was showed that abnormal changes in DJ-1 expression can be detected during IR injury in various tissues, such as heart and brain, suggesting that DJ-1 abnormality is related to IR injury[5-7]. MicroRNA

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is an endogenous, non-coding, single-stranded, and small-molecule RNA of about 22-25 nucleotides in eukaryotes. It complementary binds to the 3'-untranslated region (3'-UTR) of the target gene mRNA to degrade mRNA or inhibit mRNA translation, thus participating in the regulation of various biological processes, such as cell survival, proliferation, apoptosis, oxidative stress, and IR injury[8-10]. MiR-122 is one of the most studied microRNAs that is closely related to IR injury in many tissues and organs[11-13]. However, there is still lack of reports about whether it plays a role in renal IR injury. Bioinformatics analysis showed that there was a targeted complementary binding site between miR-122 and the 3'-UTR of DJ-1 mRNA. This study investigated the impact of miR-122 in regulating DJ-1-PTEN/PI3K/AKT signaling pathway and acute renal IR injury.

Materials and Methods

Main Reagents and Materials

Healthy adult male Sprague-Dawley (SD) rats (6-8 weeks, weight 220-240 g) were purc[illegible] from Beijing Weitong Lihua Experiment[illegible] imal Technology Co., Ltd. (Beijing, China[illegible] renal tubular epithelial cells RRTEC were [illegible] chased from Shanghai Zibosheng Biotechnolog[illegible] Co., Ltd. (Shanghai, China). Dulbec[illegible] modified eagle medium F12 (DMEM/F12) [illegible]nd fetal bovine serum (FBS) were pu[illegible]sed [illegible] Gibco BRL. Co. Ltd. (Rockville, [illegible], US[illegible] anti-rat DJ-1, PTEN, and ho[illegible]xidase (HRP) labeled Goat anti-R[illegible]bbit [illegible]) polyclonal antibodies were [illegible]hased [illegible]am Biotech. (Cambridge, [illegible]USA). Rabbi[illegible]rat AKT, p-AKT, and β-[illegible]clonal antibodies were purchased fro[illegible] Technology Inc. (Danvers, MA, USA). C[illegible]e II was purchased from [illegible]ma-Aldrich (S[illegible]uis, MO, USA). Transfe[illegible] reagent Lipofectamine 2000 was purchase[illegible]om In[illegible]gen/Life Technologies (Carlsbad, [illegible]SA[illegible]imeS[illegible]t™ RT reagent kit was purc[illegible] TaK[illegible] (Dalian, China). Luciferase activ[illegible]it was purchased from [illegible] (Mad[illegible]I, USA). miR-NC, miR[illegible], miR-122 inhibitor, and miRNA pri[illegible] we[illegible] from Ribobio (Guangz[illegible]Chin[illegible]iferase reporter plasmid wa[illegible]d from Changsha Youbao Biological (Chang[illegible]a). Apoptosis and ROS detection kits were [illegible]d from BioVision (Mountain View, CA, US[illegible] Malondialdehyde (MDA) and catalase (CAT) were purchas[illegible]yotime (Jiangsu, China). mRNA pri[illegible] were [illegible]sed from Sangon (Shanghai, [illegible]). Rats we[illegible]ed for all experiments, an[illegible] procedures [illegible]ere approved by the Anima[illegible] Commi[illegible] of Affiliated Hospital of Jining [illegible]U[illegible]rsity (Jining, Shandong, [illegible]a).

Rat Renal IR [illegible]el E[illegible]lishment

The rats we[illegible]2 h b[illegible]e operation and anesthetized [illegible]perit[illegible]l injection of 10% chlora[illegible]drate ([illegible]ch, St. Louis, MO, USA[illegible]3 cm incision [illegible]made in the middle of th[illegible]en. The left renal portal was exposed [illegible] renal pedicle was released. The b[illegible]teral re[illegible]ries were isolated and cla[illegible] with an art[illegible] clamp. After clamping f[illegible] min, the arterial clamps were removed and [illegible] renal arteries were restored to establish renal [illegible]njury model[illegible]e rat in sham group only re[illegible]d renal art[illegible]separation without clamping.

[illegible]idneys of rats were harvested and [illegible]all pieces. Then the tissue was digested with 0.1% type II collagenase for 30 min. The tissue debris was removed by filtration. The [illegible]sion was centrifuged and cell pellets [illegible]ected. The cell pellets were re-suspended in DCFH-DA diluted with serum-free 1640 medium (1:1000) at 37°C and avoid of light for [illegible]0 min. After washed by phosphylated-buffered [illegible]ine (PBS), the ROS content was detected using Beckman Coulter FC500 MCL flow cytometry (Beckman Coulter Inc., Brea, CA, USA).

Renal Function Test

The tail vein blood was collected at 20 h after the operation. After centrifugation at 400 ×g for 5 min, the upper serum was separated to test BUN and SCr levels using an automatic Olympus AU2700 biochemical analyzer (Olympus, Tokyo, Japan).

Caspase-3 Activity Detection

According to the instructions of the kit, the pNA standard product is diluted in concentration gradient to prepare standard products. The tissue was smashed to prepare homogenate. The homogenate was added with Caspase lysis buffer on ice for 20 min and centrifuged at 12000 ×g and 4°C for 10-15 min. Next, the supernatant was taken to a new 1.5 ml centrifuge tube and quantified by the BCA kit. A total of 65 µl assay buffer, 25 µl lysate supernatant, and 10 µl Ac-DEVD-pNA (2 mM) were added to a 96-well plate and incu-

bated for 2 h at 3°C. When the color change was obvious, the plate was measured at 405 nm on a microplate reader (Awareness, Laurinburg, NC, USA). The relative enzyme activity was calculated based on A405 in the experimental group/ A405 in the control group × 100%.

MDA and CAT Enzyme Activities Detection

At 20 h after operation, the renal tissues were collected to test MDA content and CAT enzyme activity to evaluate the oxidative stress in rat kidney.

IR Treatment

For I/R treatment, the cells were cultured in low glucose serum free DMEM/F12 to simulate ischemic condition. Next, the cells were maintained in incubator with 5% CO_2 and 95% N_2 to simulate hypoxic condition. The cells were changed to routine medium after 12 h and further cultured in normal condition for 12 h. RN-c cells in logarithmic phase were divided into four transfection and treatment groups, including antagomir-NC and antagomir miR-122 groups. [illegible] 72 h of transfection, the cells were treate[illegible] intervention as abovementioned. The cells [illegible] collected for detection.

Flow Cytometry Detection of Cell Apoptosis

The cells were re-suspended [illegible]00 [illegible]inding buffer and incubated in 5 µl A[illegible]xin V[illegible] 5 µl propidium iodide (PI) [illegible] for [illegible] min. Next, the cells were [illegible]dded [illegible] PI and tested on CytoFLEX flo[illegible]ytomet[illegible]man Coulter Inc., Brea, C[illegible]SA) to evalu[illegible] cell apoptosis.

Flow Cytometry [illegible]etection [illegible] ROS Content

Cells were [illegible]ed twice in PBS and digested with 0.25% [illegible]psin ([illegible]gen Biotech Co. Ltd., Beijing, C[illegible] Aft[illegible]entrif[illegible]d at 300×g for 5 min, the c[illegible]ncub[illegible] in 0.1% DCFH-DA probe at 37[illegible] After resuspension in 50[illegible]S, the [illegible]ere tested using a Beck[illegible]00MCL flo[illegible] cytometer (Beckman C[illegible] Inc.[illegible]USA).

Du[illegible]erase Reporter Gene Assay

HE[illegible]lls were seeded in 24-well plate (Corning C[illegible]orning, NY, USA) for 24 h. Then pGL3-DJ[illegible]-3'-UTR-wt (or pGL3-DJ-1-3'-UTR-mut), miR-122 mimic (or [illegible]iR-122 inhibitor), and pRL-null Re[illegible] luci[illegible]ere co-transfected to HEK293[illegible]lls using L[illegible]00 (Invitrogen/Life Techn[illegible]s, Carlsbad[illegible]CA, USA). The luciferase ac[illegible] detect[illegible]fter cultured for 48 h.

Quantitative R[illegible] Time PCR (qR[illegible]CR)

Total RNA w[illegible]xtract[illegible]sing PrimeScript™ RT reagent Ki[illegible]R047[illegible]aKaRa, Dalian, China) and [illegible]or P[illegible] reaction. The reaction sy[illegible] conta[illegible]×SYBR Green Mixture, [illegible]µl of 5 µm/l p[illegible]ers, 1 µl complementary [illegible]DNA), and ddH_2O. The reverse transc[illegible]o[illegible]n was 50°C for 15 min and 85°C [illegible] 5 min. [illegible] reaction was composed of [illegible] pre-denatura[illegible] for 5 min, followed by [illegible]cles of 95°C for 15 s and 60°C for 1 min. Re[illegible]me PCR was performed on Bio-Rad CFX96/[illegible]X connect ([illegible]Rad Laboratories, Hercules, [illegible] USA) to [illegible] the relative expression. The p[illegible] used were as follows. miR-122P_F: 5'-[illegible]GACAATGGTGTTTG-3', miR-122P_R: 5'-GCGAGCACAGAATTAATACGAC-3'; U6P_F: 5'-CGCTTCGGCAGCACATATAC-3', [illegible]TCACGAATTTGCGTGTCAT-3'; DJ-[illegible]AGCAGAGGAAATGGAGACG-3', DJ-[illegible]$_R$: 5'-GCCAACAGAGCAGTAGGAC-3'; β-actinP_F: 5'-TCTACAATGAGCTGCGTGTG-3', β-actinP_R: 5'-ATCTCCTTCTGCATCCTGTC-3'.

Western Blot

The total protein was extracted by radioimmunoprecipitation assay (RIPA) from cells. A total of 40 µg protein were separated by 10% sodium dodecyl sulphate-polyAcrylamide gel electrophoresis (SDS-PAGE) and transferred to membrane at 300 mA for 90 min. Next, the membrane was blocked and incubated in primary antibody at 4°C overnight (DJ-1, PTEN, AKT, p-AKT, and β-actin at 1:2000, 1:1000, 1:2000, 1:1000, and 1:10000, respectively). Next, the membrane was incubated in secondary antibody (1:20000) for 60 min after washed by PBS Tween-20 (PBST, Tiangen Biotech Co. Ltd., Beijing, China) for three times. At last, the protein expression was detected by enhanced chemiluminescence (ECL) (Amersham Biosciences, Piscataway, NJ, USA).

Statistical Analysis

All data analyses were performed on SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were depicted as mean ± standard deviation and compared by Student'

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t-test. Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data between groups. $p < 0.05$ was considered as statistical significance.

Results

MiR-122 Targeted Regulated DJ-1 Expression

MicroRNA.org online prediction showed the targeted binding site between miR-122 and 3'-UTR of DJ-1 mRNA (Figure 1A). Dual luciferase assay revealed that miR-122 mimic or inhibitor significantly declined or increased the relative luciferase activity of HEK293T cells transfected by pGL3-DJ-1-3'-UTR-wt, while it exhibited no statistical impact on the luciferase activity in HEK293T cells transfected by pGL3-DJ-1-3'-UTR-mut (Figure 1B), indicating the regulatory relationship between miR-122 and DJ-1 mRNA.

Significant Renal Injury, Enhanced Oxidative Stress and Caspase 3 Acti[illegible] in I-R Rats

The renal function test showed that com[illegible] with the Sham group, the levels of BUN and [illegible] in the serum of the IR model increased signi[illegible]cantly, indicating that the renal fu[illegible]tion of the IR rat model was significantly i[illegible] (Table I). DCFH-DA staining revealed [illegible] RO[illegible]ontent in kidney tissue of IR model [illegible] was [illegible] higher than that of Sham gr[illegible]A, p [illegible] 0.05). Compared with the [illegible]ham [illegible]e MDA content was apparently e[illegible]ted (Fi[illegible] $p <$ 0.05), while the CAT [illegible]ity was sig[illegible]tly decreased in the bra[illegible] of the IR model (Figure 2C, $p < 0.05$[illegible]pe[illegible]metric results demonstrated that t[illegible] caspase[illegible]ity in the renal tissue of [illegible] IR model wa[illegible]nificantly higher than th[illegible] the Sham group (Figure 2D, $p < 0.05$).

MiR-122 a[illegible]bno[illegible]lly Expressed in the Renal T[illegible]of [illegible]at

qRT[illegible]emons[illegible]at the expression of miR[illegible] renal tiss[illegible] of the IR group was sig[illegible]antly [illegible]n that of the Sham group, a[illegible]expr[illegible] was significantly higher tha[illegible]e 10 h (Figure 3A, $p < 0.05$). Compared [illegible] Sham group, the expression of DJ-1 mRN[illegible] renal tissue of the IR group was markedly [illegible]duced, and its expression level [illegible]t 20 h was apparently lower than that at the 10 h ([illegible]gure 3B, $p < 0.05$). Western blot showed that [illegible]he expression of DJ-1 protein in the renal tissue of rats in IR group was significantly lower than that in Sham group at the same time (Figure 3C, $p < 0.05$).

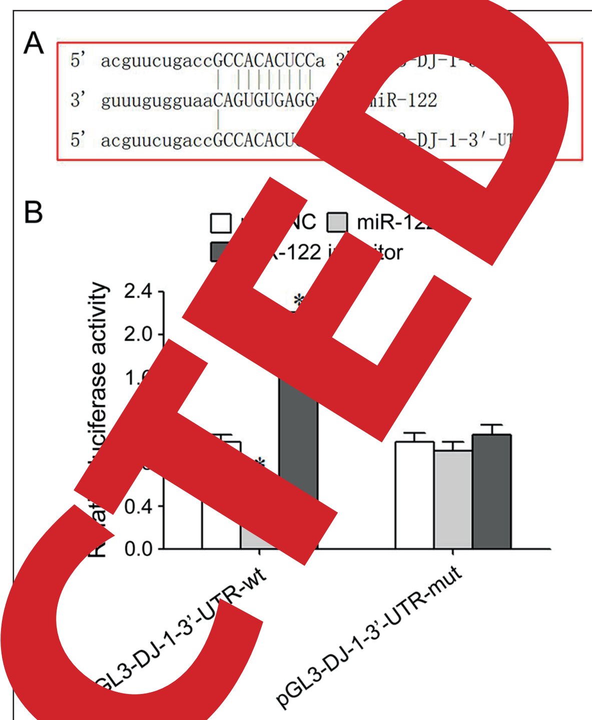

Figure 1. MiR-122 targeted regulated DJ-1 expression. ***(A)*** The binding site between miR-122 and the 3'-UTR of DJ-1 [illegible]ual luciferase assay. *$p < 0.05$, compared with

Down-Regulation of miR-122 Attenuated Tubular Epithelial Cell Apoptosis Induced by IR

qRT-PCR showed that compared with the control group, the expression of miR-122 was significantly increased (Figure 4A), whereas the expression of DJ-1 mRNA was obviously declined in rat RRTEC cells treated by IR (Figure 4B, $p < 0.05$). Transfection of miR-122 inhibitor on the basis of I-R treatment apparently reduced

Table I. Renal function comparison.

Index	BUN (mmol/l)	SCr (μmol/l)
Sham group (n = 5)	9.32 ± 1.57	26.61 ± 3.75
IR group (n = 5)	26.73 ± 5.11*	73.59 ± 8.23*

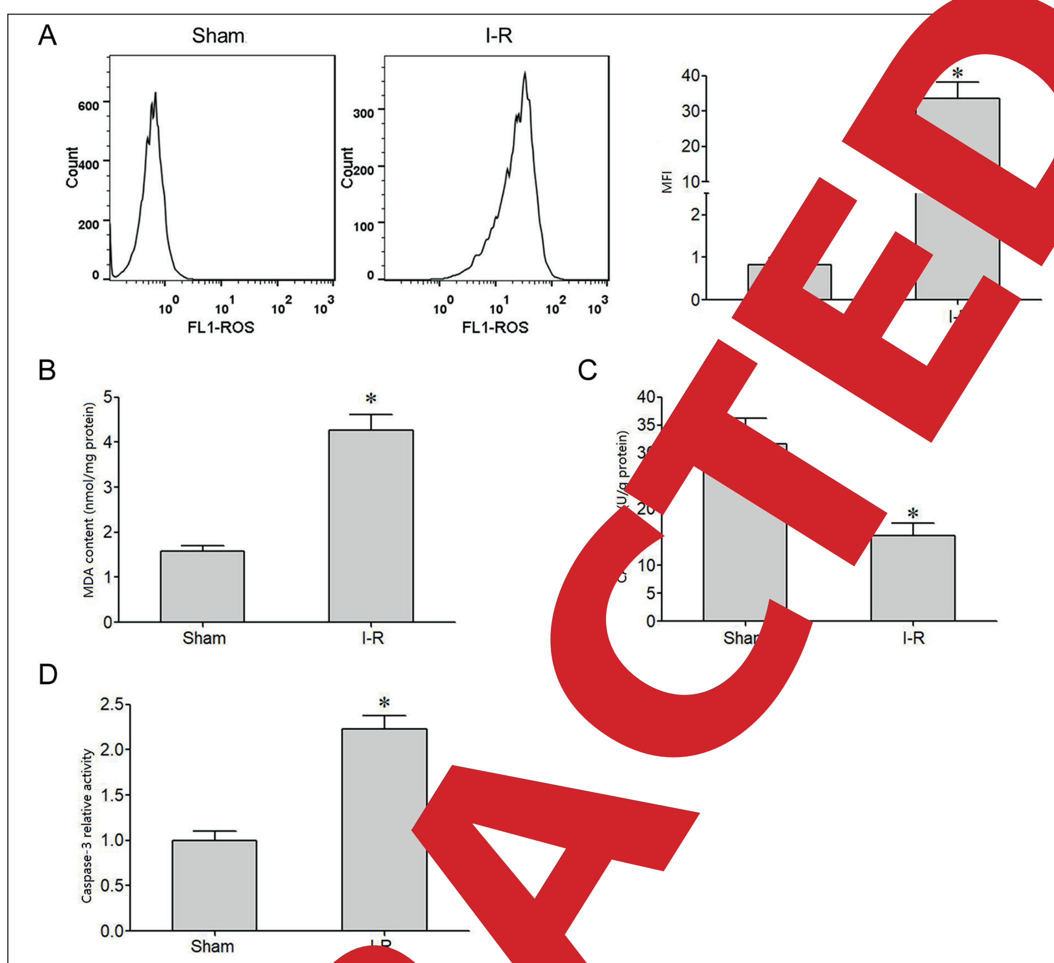

Figure 2. Significant renal injury enh[...]ive stress an[...]aspase 3 activity in I-R rats. ***(A)*** Flow cytometry detection of ROS content in renal tissue. ***(B)*** [...] cont[...] in renal tissue. ***(C)*** CAT activity detection in renal tissue. ***(D)*** Spectrophotometry detection of ca[...] acti[...]e. $*p < 0.05$, compared with Sham group.

the expression of miR-1[...]nd enha[...] expression of DJ-1 mRN[...] Western blot [...]sis exhibited that compa[...] the control group, IR treatment signi[...]ntly [...] the expression of DJ-1 protein, increased the expression of PTEN protein, and declined the expression of p-AKT protein in RRTEC cells (Figure 4C, $p < 0.05$). Down-regulation of miR-122 ex-

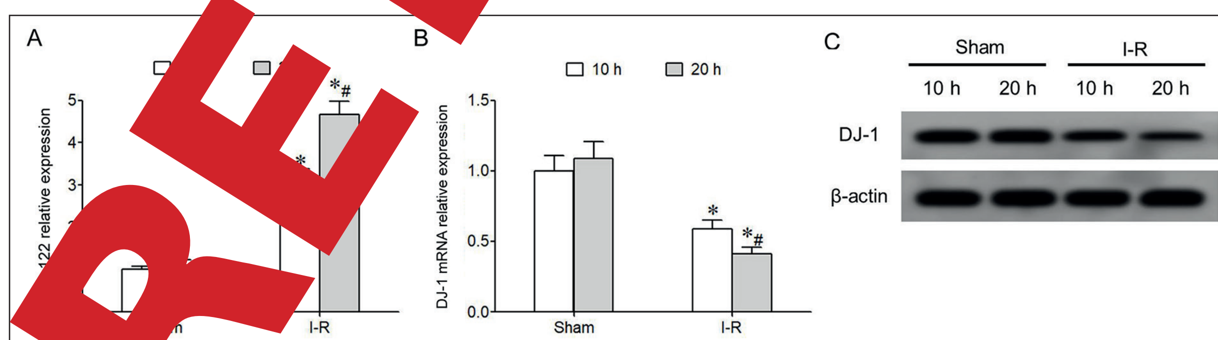

Figure 3. [...] and DJ-1 abnormally expressed in the renal tissue of IR rat. ***(A)*** MiR-122 expression in renal tissue detected by qR[...] ***(B)*** DJ-1 mRNA expression in renal tissue detected by qRT-PCR. ***(C)*** DJ-1 protein expression in renal tissue detected by [...]estern blot. $*p < 0.05$, compared with Sham group. $^{\#}p < 0.05$, compared with 10 h.

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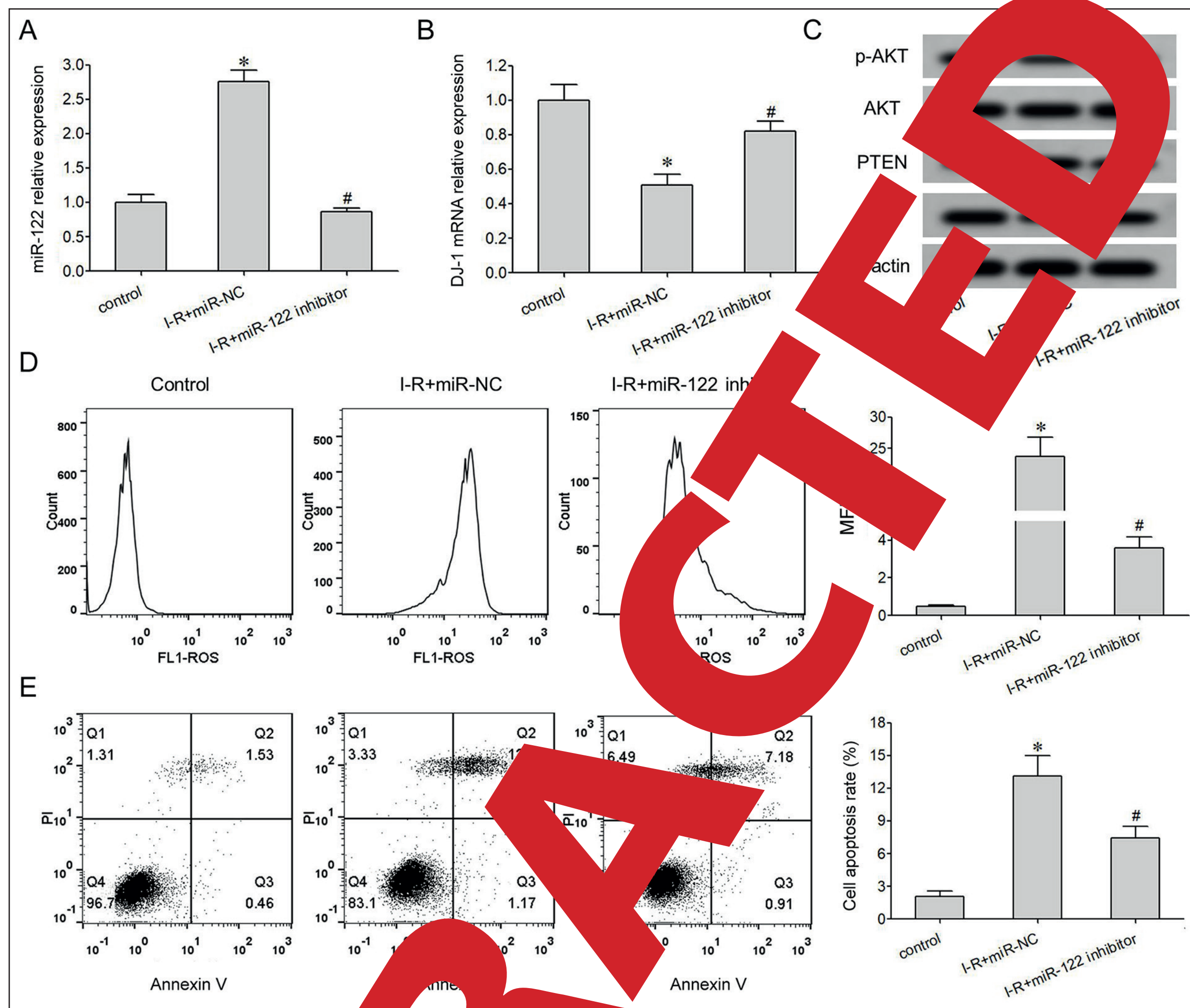

Figure 4. Down-regulation of mi ithelial cell apoptosis induced by IR. ***(A)*** MiR-122 expression detected by qRT-PCR. ***(B)*** DJ-1 mR n detected by qRT-PCR. ***(C)*** DJ-1 protein expression detected by Western blot. ***(D)*** ROS content detected low cy ***(E)*** Cell apoptosis detected by flow cytometry. $^*p < 0.05$, compared with control. $^\#p < 0.05$, compared w R+miR-NC

pression significantl elevat xpression of DJ-1 protein uced PTEN ex sion, and enhanced PI3K KT pathway in RRTEC cells (Figure 4C, 0.05) ow cytometry showed that after t ctio miR inhibitor, the ROS conten ficant educed, and the apoptosis rate wa attenuated under IR trea (Figure $p < 0.05$).

ssion

Dur 3K/AKT signaling pathway activation, PI3 zes the phosphorylation of its substrate phosp tidylinositol 4,5-trisphosphate (PIP2) to phosphatidylinositol 3,4,5- Phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 recruits AKT from the cytoplasm to transmembrane and phosphorylates AKT under the action of phosphoinositide dependent protein kinase (PDK). Phosphorylated AKT continue to regulate the expression and function of downstream proteins that play a key role in the regulation of cell proliferation, cycle, and apoptosis[14,15]. The DJ-1/PARK7 (Parkinson gene 7) gene is located on human chromosome 1p36.2-36.3. It is about 24 kb in length and encodes a protein with a molecular weight of 21 kD composed of 189 amino acids[4]. DJ-1 is a negative regulator of PTEN that plays a role in inhibiting the expres-

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sion and function of PTEN and enhancing the transduction activity of PI3K/AKT signaling pathway[4]. It was showed that abnormal changes in DJ-1 expression can be detected during IR injury in various tissues, such as heart and brain, suggesting that DJ-1 abnormality is related to IR injury[5-7]. MiR-122 is one of the most studied microRNAs that is closely related to I-R damage in many tissues and organs[11-13]. However, there is still lack of reports about whether it plays a role in renal IR injury. Bioinformatics analysis showed that there was a targeted complementary binding site between miR-122 and the 3′-UTR of DJ-1 mRNA. This study investigated the impact of miR-122 in regulating DJ-1-PTEN/PI3K/AKT signaling pathway and acute renal IR injury. Dual luciferase reporter gene assay showed that transfection of miR-122 mimic significantly reduced relative luciferase activity in pGL3-DJ-1-3′-UTR-wt transfected HEK293T cells. Whereas transfection of miR-122 inhibitor obviously enhanced the activity of relative luciferase, indicating that there is a targeted regulatory relationship between miR-122 and DJ-1. The results of rat kidney [illegible] examination showed that the MDA cont[illegible] apparently increased, CAT enzyme activi[illegible] markedly reduced, and ROS content was [illegible]nificantly elevated, indicating that IR modeli[illegible] can lead to obvious renal oxidative [illegible]ss injury. During IR injury, the expressi[illegible]iR-122 was significantly increased, [illegible] th[illegible]xpression of DJ-1 was significan[illegible]ecre[illegible] time dependence. It indicat[illegible]reased expression of miR-122 m[illegible]y be[illegible]ibitor of DJ-1 expression and a [illegible]motion [illegible] IR injury in kidney tissu[illegible]kbari et al[illegible]ved that the expression o[illegible]2 in serum of liver IR model rats was [illegible]re[illegible] times higher than that of control. Farid et [illegible]rted that compared with [illegible]thy controls, t[illegible]xpression of miR-122 in [illegible]um of patients with hepatic IR injury increa[illegible] seve[illegible]enths, and was related to the exte[illegible]liv[illegible]jury. [illegible] Caster et al[13] revealed tha[illegible]essio[illegible] miR-122 in the serum of rats wa[illegible]y increased (nearly 100[illegible]fter 4 [illegible]el establishment of liver [illegible] The expr[illegible]sion level of miR-122 wa[illegible]rrela[illegible] degree of liver function d[illegible] I[illegible]kul et al[18] also observed a [illegible] increase in miR-122 expression during [illegible]R injury. Our results found that the abnorm[illegible]er-expression of miR-122 may be involved i[illegible]he process of renal IR injury, which was similar to the r[illegible]kbari[16] and Van Caster[13]. Over-ex[illegible] [illegible]122 may be a promoting fa[illegible] for IR i[illegible] It was showed that miR-12[illegible]ibitor signi[illegible]tly down-regulated the up-[illegible]n of [illegible]122 expression, elevated the d[illegible] DJ-1 expression, reduce[illegible]tracellula[illegible]roduction, and attenua[illegible]apop[illegible]is rate [illegible]uced by IR. Mard et al[illegible] [illegible]mons[illegible]ed th[illegible] Cr and ZnSO4 can, enh[illegible]oxid[illegible]capacity of liver and re[illegible] [illegible]injur[illegible]rough inhibiting miR-[illegible] expres[illegible]t al[20] reported that low [illegible]perature pre[illegible]ment can reduce the exp[illegible] miR-122 and IR injury to liver cells. [illegible] exhibited that overexpression o[illegible]miR-122 [illegible]favorable factor in IR in[illegible] while correc[illegible] the expression of miR-[illegible] may play a role in alleviating IR injury.

[illegible] the process of renal IR injury, in addi[illegible] to the abno[illegible]lly increased expression of [illegible]-122, dow[illegible]gulation of DJ-1 is also an [illegible]ular event. Our *in vitro* results sh[illegible]own-regulation of miR-122 expression can protect IR injury by up-regulating [illegible] expression, inhibiting PTEN expression, [illegible]cing the activity of PI3K/AKT pathway [illegible]educe apoptosis and oxidative stress. [illegible]jiri et al[21] observed that Cyclosporine A (CSA) can up-regulate DJ-1 expression and [illegible]educe IR injury in rat neurons. Yang et al[5] [illegible]hibited that sodium phenylbutyrate (SPB) can up-regulate the expression of DJ-1 and reduce I-R-induced neuronal injury. Shen et al[22] found that IR treatment on NRK-52E cells significantly reduced DJ-1 expression, obviously decreased cell viability, attenuated SOD expression, and elevated MDA content. Elevated DJ-1 can reduce IR injury. It confirmed that decreased expression of DJ-1 is a detrimental factor in IR injury, which was in accordance with our results. At present, there is no report on the relationship between miR-122 and renal IR injury. This study found that the increase of miR-122 is related to renal IR injury, and the abnormal expression of DJ-1 plays a role in the process. In this study, we integrated the relationship between miR-122 and targeted DJ-1, revealing the role of miR-122 in inhibiting the expression of DJ-1, decreasing the activity of PTEN-PI3K/AKT pathway, and promoting renal IR injury. However, whether miR-122 plays a role in the expression of DJ-1 and renal IR injury in animals still requires further experimental research.

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Conclusions

We revealed that MiR-122 increased significantly, while DJ-1 declined significantly during renal IR injury. Down-regulation of miR-122 markedly elevated DJ-1, enhanced PTEN/PI3K/AKT pathway activity, and inhibited apoptosis and ROS generation in rat renal tubular epithelial cells to alleviate IR injury.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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RETRACTED