Mir10a aggravates the ischemia-reperfusion kidney injury by inhibiting the PI3K/AKT signaling pathway

CURRENT STATUS: UNDER REVISION

BMC Nephrology  BMC Series

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DOI: 10.21203/rs.2.23453/v1

SUBJECT AREAS
Urology & Nephrology

KEYWORDS
miR-10a, PIK3CA, PI3K/AKT signaling pathway, renal ischemia reperfusion
Abstract

Background

To investigate the effect of miR-10a on PI3K/AKT signaling pathway. The ischemia-reperfusion injury models of rats were simulated in vivo.

Methods

RT-PCR was used to test the expression of miR-10a. The serum creatinine and urea nitrogen levels were determined. The pathological changes and the apoptosis of renal cells were observed. The model of HK-2 cells with hypoxia-reoxygenation was established in vitro. The cell proliferation and apoptosis rate were tested by CCK8, clone formation and flow cytometry, respectively. The apoptosis-related proteins and PIK3CA and PI3K/AKT signaling pathway-related proteins were detected by Western blot both in vivo and intro. The dual luciferase assay was used to verify whether PIK3CA is a target gene of miR-10a. PIK3CA gene was over-expression or silenced. The transfection efficiency was verified by RT-PCR and the above experiments were repeated.

Results

Compared with I/R group, miR-10a RNA was significantly increased in renal tissue of miR-10a group, serum Cr and BUN levels, and renal injury score and apoptosis index were significantly increased, while the expression of PI3K/AKT signaling pathway-related proteins were significantly inhibited. However, the indicators above were contrary in anti-miR group. In comparison with H/R group, miR-10a RNA expression was remarkably increased in miR-10a cells and the cell proliferation was inhibited. The apoptosis rate was increased and the expression of PI3K/AKT signaling pathway-related proteins were down-regulated. However, the indicators above were contrary in anti-miR group.

Conclusion

miR-10a can aggravate the ischemia-reperfusion-induced renal injury in rats by targeting PIK3CA and inhibiting PI3K/AKT signaling pathway.

Background

Kidney transplantation is often accompanied with unavoidable acute renal failure, the main cause of which is considered to renal ischemia-reperfusion injury (IRI), in addition, it is also considered to be an
adverse effect on different survival rates after kidney transplantation [1,2]. Renal tubular and hemodynamics damage often were accompanied by an inflammatory response, which can induce a complicated reaction that cause renal IRI [3]. The cell metabolism and inflammation would change when IRI happens, which lead to the production of free radicals and cell apoptosis. Then the renal tubular cells will detach from the basement membrane and fall off into the urine subsequently [4]. The IRI happens when the blood flow is recovered from the ischemic situations, which have continued for a period of times. Furthermore, it can also induce an increase in morbidity and mortality accompanied by cardiopulmonary bypass, myocardial infarction, gut ischemia and stroke [5].

microRNAs are a kind of small non-coding RNAs that can regulate mRNA expression at the post-transcriptional level. And the regulating mode is directly couple on 3’un-translated region (3’UTRs) of target gene message RNA (mRNA) [6,7]. Moreover, microRNAs can affect a extensiveness of signaling pathways, more importantly, the dysfunction of miRNA regulation may be relevant to the happening and progress of tumor and lots of other problems [8]. miR-10a is a member of miR-10 family, generally featured by obvious heterogeneity [9,10]. Moreover, the previous studies showed that miR-10a played a key role in the process of protein synthesis [11], programmed cell death [12], and cancer [13]. But the underlying mechanism of miR-10a in I/R injury models has not been reported.

As a class of important stable signaling transduction molecule, phosphatidylinositol 3-kinase (PI3K) family plays a great important role in activating its downstream signal factor, serine/threonine kinase Akt (also famous as protein kinase B, PKB), which regulates the physiological progress of cell life activity and different biological reactions including oxidative stress, chemotaxis reaction inflammation and apoptosis [14,15]. Previous studies [16] have found that I/R can cause the up-regulation of PI3K/Akt, which can protect the injury by accelerating the proliferation and cytoactivity of renal tubular epithelial cells. But the upstream and the related regulatory mechanisms are elusive in I/R injury models.

In the present work, an ischemia/reperfusion-induced IRI rat model and a hypoxia-induced HK-2 cell model was established in vivo and in vitro. And the studies were performed to investigate the effect of miR-10a targeting PIK3CA on PI3K/AKT signaling pathway and renal ischemia-reperfusion injury by
targeting PIK3CA.

Methods

Experimental animals

Healthy SPF grade forty-eight male Sprague-Dawley rats, 8-10 weeks old and weighing 220±20g, were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd., animal production license number: SCXK (Lu) 2014-0007. All animals were fed in a controlled environment at 22-24 °C and a relative humidity of 50-60% as well as under a 12-h dark/light cycle with free access to food and water.

Animal grouping and IRI model construction

Twelve rats were randomly divided into two groups according to the random number table method: sham operation group (Sham) and renal ischemia reperfusion group (I/R) (n=6). The rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (50 mg/kg). The right kidney was taken and then the left renal artery was clamped for 45 min after the mid-abdominal incision [17,18]. The blood flow was fully restored before the abdomen being closed after removal of the arterial clip. The abdominal surgery was performed in the sham group. To verify the effect of miR-10a on IRI rats, 36 rats were randomly divided into 6 groups (n=6): Sham group, Renal ischemia-reperfusion group (I/R), miR-10a agonist group (miR-10a), miR-10a agonist negative control group (miR-NC), miR-10a inhibitor group (anti-miR), miR-10a inhibitor negative control group (anti-NC). The rats in Sham and I/R groups were injected the same amount of normal saline into the tail vein 1 h before surgery. The rats in miR-10a and miR-NC groups were injected miR-10a agonist 10mg/kg and miR-10a agonist negative control 10mg/kg into the tail vein 1 h before surgery, respectively. And the rats in anti-miR and anti-NC groups were injected miR-10a inhibitor 10mg/kg and miR-10a inhibitor negative control 10mg/kg into the tail vein 1 h before surgery, respectively.

The rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (50 mg/kg) and sacrificed by dislocation after postoperatively 24 hours. 5 ml of blood was taken from the abdominal aorta. The kidneys were partially stored in a refrigerator at -80 ° C and partially placed in 4% paraformaldehyde for 24 h after being taken out. Then the fixed tissue was embedded in paraffin.
Cell Culture and Model Construction

Human renal tubular epithelial cells HK-2 were purchased from Shanghai Institute of Cell Research, Chinese Academy of Sciences, which was cultured in DMEM/F12 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin (GIBCO, Invitrogen, Grand Island, NY, USA). The medium was cultured in a constant temperature incubator (Thermo, USA) with 37°C, 5% CO₂. The logarithmic growth cells were planted in complete medium at a density of 1 x 10⁵/ml and 24h before the chemical treatment. The cells are divided into 2 groups: a) Control group: cells were cultured for 27 hours; b) Hypoxia/reoxygenation group (H/R): anoxic 24h (5% CO₂, 1% O₂, 94% N₂), reoxygenation 3h (5% CO₂, 21% O₂, 74% N₂).

miR-10a gene transfection and grouping

The cell line was planted into a six-well plate 24h before transfection, and when the cell fusion was up to 40%, the transfection was conducted as following: Lipofectamine TM2000 (Invitrogen, Carlsbad, CA) and plasmid (GenePharma, Shanghai, China) were used and the detailed procedures were according to the previous study [19]. According to different treatment methods, the H/R group cells were divided into 5 groups: Hypoxia/reoxygenation group (H/R), miR-10a mimetic group (miR-10a), miR-10a mimetic negative control group (miR-NC), miR-10a Inhibitor group (anti-miR), miR-10a inhibitor negative control group (anti-NC). The H/R group was without transfection. The miR-10a group was transfected with miR-10a mimic (sense, 5'-CAAAUUCGGAUCUACAGGGUAUU-3' and anti-sense, 5'-UACCCUGUAGAUCCGAUUUGUG-3'). The miR-NC group was transfected with unrelated siRNA as a negative control (sense, 5'-UUCUCGAACGUGUCAGGUTT-3' and anti-sense, 5'-UACCCUGUAGAUCCGAUUUGUG-3'). The anti-miR group was transfected with miR-133a-3p inhibitor, (5'-CAC AAAUUCGGAUCUACAGGUA-3'). The anti-NC group was transfected with unrelated siRNA as a negative control(5'-CAGUACUUUUGUGUAGUACAA-3').

PIK3CA gene transfection and grouping

The PIK3CA gene was transfected into HK-2 cells using LipofectamineTM 2000, siRNAs against Raptor, which was purchased from Dharmacon (Thermo Scientific, Brookfield, WI, USA). To further verify
whether PIK3CA is a target gene of miR-10a, cells were grouped into: control group, hypoxia/reoxygenation group (H/R), miR-10a mimic group (miR-10a), PIK3CA silencing group (si-P), PIK3CA silencing negative control group (si-NC), miR-10a mimic + PIK3CA overexpression group (miR + P), miR-10a mimic + PIK3CA overexpression negative control group (miR + P-NC). The transfection efficiency was detected by RT-PCR, and the above experiments were repeated for verification.

**Renal function test**

The blood was stewed for 30 min, and then was centrifuged at 3000 r/min for 10 min. The serum was taken after the centrifugation. Serum creatinine (Cr) and urea nitrogen (BUN) levels were measured by an automatic biochemical analyzer to compare differences between different groups of renal function.

**Pathological changes were detected by HE staining**

Paraffin wax was serially sliced to a thickness of 5 μm. The sections were conventionally dewaxed with xylene and hydrated by various stages of ethanol. The section was stained for 5 min in hematoxylin (Solarbio, Beijing, China), and then rinsed with tap water. The section was differentiated for 30s in hydrochloric acid ethanol and then soaked in tap water for 15 min. The section was placed in Eosin stain (Solarbio, Beijing, China) for 2 min after that. Lastly, the section was routinely dehydrated, transparent and sealed. Renal histopathological changes were observed under a ×400 optical microscope (Olympus Model BX51, Olympus, Japan). A five-point quantitative scoring method [20] was used to blindly measure the pathological changes of renal tissues according to the degree of renal tubular necrosis, swelling of renal tubular epithelial cells, vacuolization and shedding: 0, <10%; 1, 10-25%; 2, 25-50%; 3, 50-75%; and 4, 75-100%.

**Renal cell apoptosis was detected by TUNEL staining**

The cell apoptosis was detected by TUNEL method using the apoptosis detection kit (batch number: ZK-8005, Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., China). The sections were randomly selected for 5 fields of view under a 400-fold optical microscope (BX50/Olympus, Japan). The apoptotic cells were brown or brownish yellow as well as had apoptotic cell morphology. Apoptotic index \( AI \) was calculated, which reflected the degree of apoptosis. \( AI = \frac{\text{number of apoptotic positive cells}}{\text{total number of cells}} \times 100\% \).
miR-10a expression in renal tissues and cells were detected by RT-PCR

Total RNA samples from the HK-2 cells and the kidney tissue was extracted according to TRIzol kit (Takara, Dalian, China) (The value of OD260/OD280 between 1.8 and 2.0 indicates the acceptable RNA purity). The cDNA was synthesised by the reverse transcription kit (Applied Biosystems, Waltham, MA, USA). RT-PCR was performed using a Mastercycler® nexus X2 (Eppendorf, Hamburg, Germany). Conditions: 95 ° C for 15 s, 60 ° C for 60 s, 72 ° C for 40 s (35 cycles). Data were processed by the 2^{-ΔΔCt} method and relative expression levels were calculated using U6 mRNA as an internal parameter.

The primers (Shanghai Shenggong Bioengineering Technology Service Co., Ltd.) are as follows: miR-10a Forward: 5′- CTGGAAAAATTCTGGGCCAA -3′. miR-10a Reverse: 5′-CCAGACTGTCTCATTGAGAAGAA -3′. U6 Forward: 5′- GACCTCTATGCCAACACAGT -3′. U6 Reverse: 5′-AGTACTTGCCTCAGGAGGA -3′. PIK3CA Forward: 5′- GCATACATTCAAGAGACC -3′. PIK3CA Reverse: 5′-CTCAGTTATCTTTCAG-3′. GAPDH Forward: 5′- TGACTTCAACAGCGACACCCA -3′. GAPDH Reverse: 5′-CACCCCTGTGTGGAGCCTGAAA -3′.

The expression of apoptosis, PIK3CA, PI3K/AKT Signaling Pathway related proteins in renal tissues and cells were detected by Western blot

The protein of cells and the kidney tissue were extracted following the kit instructions, and the concentration of protein sample was measured by a BCA protein quantification kit (23225, Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham, USA). Each group of samples was loaded with 40 μg and separated by SDS-PAGE electrophoresis (Mini-Protean-3, Bio-Rad, Hercules, CA, USA). Then the samples were transferred to PVDF membrane (Millipore, Massachusetts, USA) for 30 min and then blocked in 5% degreased milk powder for 1 h. The primary antibody of each protein was diluted with 5% BSA. And the rabbit anti-rat PIK3CA (1:500, orb228203, Biorbyt, Cambridge, UK), PI3K (1:500, orb137259, Biorbyt, Cambridge, UK), p-PI3K (1:500, orb338965, Biorbyt, Cambridge, UK), AKT (1:500, orb213545, Biorbyt, Cambridge, UK), p-AKT (1:500, orb222951, Biorbyt, Cambridge, UK), Bax(1:500,
orb224426, Biorbyt, Cambridge, UK; Bcl-2 1:500, orb228150, Biorbyt, Cambridge, UK; caspase-3 1:500, orb10231, Biorbyt, Cambridge, UK; β-actin (1:2000, orb178392, Biorbyt, Cambridge, UK) polyclonal antibody were diluted with TBST solution containing 3% bovine serum albumin. All the antibodies were reacted overnight at 4°C and goat anti-rabbit Ig G (1:1000, ab6721, Abcam, UK) were incubated for 1h. The PVDF membrane was detected by ECL chemiluminescence, grayscale scanning and quantification by Image J software (NIH). Protein expression levels were normalized to β-actin.

**The proliferative activity of cells after transfection was detected by CCK8**

Cells at logarithmic growth phase were planted into 96-well plates at a density of 2 x 10^4 cells/ml with 100 μl per well, which were cultured in a 37 °C, 5% CO₂ incubator for 24 h, 48h, 72h, and 96h. 10 μl CCK-8 solution (Tongren Chemical Research Institute, Japan) was added into each well for another 4h. The blank control well was adjusted to zero, and the absorbance (OD) value of each well was measured at 450 nm by enzymatic marker.

**Clone formation**

The logarithmic growth phase cells were planted into six-well plate at a density 250 cells/ml with the volume of 2 ml. The cells were cultured in a 37 °C, 5% CO₂ environment for 2 to 3 weeks. And fresh medium was changed every three days. The cells were fixed with methanol and stained by 1ml Giemsa solution per well for 30 min, then washed twice in ultrapure water. Lastly, the water around the dish was absorbed by filter paper. The picture was taken by a camera.

**Cell apoptosis was detected by flow cytometry**

The cells were cultured for 24 h, then collected and resuspended once with pre-cooled 1×PBS (4°C). The cells were centrifuged at 1000 rpm for 5-10 minutes. Then 300 μl of 1× Binding Buffer was used to suspend the cells. 5μl of Annexin V-FITC was added into cells. And the cells were incubated for 15 minutes at room temperature. 5 minutes before the detection, the 5μl of PI staining was added and then 200μl of 1×Binding Buffer was added. Samples were finally detected by flow cytometry (Beckman Coulter, Brea, CA, USA) and analyzed by Cell Quest software (BD Bioscience, San Diego, CA).
**Dual luciferase reporter assay**

The overall length 3’-UTR of PI3K catalytic subunit α (PIK3CA) was subcloned into a pMir-Target luciferase vector that promoted by a cytomegalovirus (CMV) promoter (Origene, Rockville, MD, USA). Mutant derivatives of the structure were fabricated by site-directed mutagenesis by the Quick-Change Kit (Stratagene, La Jolla, CA, USA). HEK293 cells were cotransfected with the luciferase structure (wild type or mutant) with miR-10a mimics or control scrambled siRNA for 48 h using Lipofectamine (Invitrogen) at a 2:1 molar ratio (miRNA mimics vs. construct reporter) according to the manufacturer’s instruction. The cells were counted and reseeded into 96-well assay plates 24 h before luciferase detection, followed by dual-luciferase assay according to the instruction (Promega, Madison, WI, USA). Luminescence was detected by a multimode microplate reader (BioTek Synergy 2; BioTek, Winooski, VT, USA).

**Statistical methods**

Data processing was performed using SPSS 19.0 statistical analysis software, and the results of data analysis were expressed as mean ± standard deviation (mean ± SD). The t-test was used for data analysis between the two groups; the one-way analysis of variance (ANOVA) was used for data analysis among multiple groups, and the LSD test was used for subsequent analysis. The difference was statistical significantly with p < 0.05.

**Results**

**Effect of miR-10a on renal function in rats**

As the results showed in the Figure 1, when comparing with the Sham group, the miR-10a RNA was greatly increased in the renal tissue of the I/R group (p < 0.05), and the serum Cr and BUN levels were also increased (p < 0.05). HE staining showed that renal was congestive and edematous. The epithelial cells of the small tube were obviously swollen. And some of the tubular epithelial cells were necrotic and shedding. Also, the inflammatory cells infiltrated in the renal interstitial and the renal injury score was significantly increased (p < 0.05) in the I/R group. In addition, the miR-10a RNA in the kidney tissue of the miR-10a group was greatly increased in contrast to I/R group. The serum Cr
and BUN levels and the renal injury score were also significantly increased (p < 0.05). But the miR-10a gene was obviously reduced in the kidney tissue of the rat in anti-miR group, the serum Cr, BUN levels and renal injury scores were also remarkably reduced (p < 0.05). The miR-10a RNA was obviously decreased in the anti-miR group than the miR-10a group. Furthermore, serum Cr, BUN levels and the renal injury score were also remarkably decreased (p < 0.05).

**Effect of miR-10a on renal cell apoptosis and expression of PI3K/AKT signaling pathway-related proteins in rats**

The effect of miR-10a on renal cell apoptosis and expression of PI3K/AKT signaling pathway-related proteins were demonstrated in the Figure 2. The apoptosis index of renal cell, the expression of apoptosis-related proteins Bax and Caspase-3 were obviously increased in the I/R group (p < 0.05) and the expression of Bcl-2, PIK3CA, p-PI3K/PI3K, p-AKT/AKT proteins were also remarkably decreased than the Sham group (p < 0.05). Compared with the I/R group, the apoptosis index of renal cell, the expression of apoptosis-related proteins Bax and Caspase-3 were outstandingly increased in the miR-10a group (p < 0.05) and the expression of Bcl-2, PIK3CA, p-PI3K/PI3K, p-AKT/AKT proteins were significantly decreased (p < 0.05). Furthermore, the apoptosis index of renal cell in the anti-miR group was significantly decreased, the expression of apoptosis-related proteins Bax and Caspase-3 was significantly decreased, and the expression of Bcl-2 protein was significantly increased. The expression of PIK3CA, p-PI3K/PI3K, p-AKT /AKT were significantly increased versus the I/R group (p < 0.05). On the contrary, when comparing with the miR-10a group, the above indicators were significantly improved in the anti-miR group (p < 0.05).

**Effect of miR-10a on proliferation of HK-2 cells**

To further verify the effect of miR-10a, HK-2 cells were used in vitro studies (Figure 3). Compared with the Control group, the expression of miR-10a RNA was greatly increased in the H/R group and the cell proliferation activity was remarkably reduced (p < 0.05). The level of miR-10a RNA was remarkably increased in the miR-10a group and the cell proliferation activity was notably reduced (p < 0.05) compared with the H/R group; in addition, the expression of miR-10a RNA was notably down-regulated in the anti-miR group and the cell proliferation activity was significantly increased (p < 0.05). Lastly,
the expression of miR-10a RNA was greatly decreased and the cell proliferation was outstandingly elevated in the anti-miR group versus the miR-10a group (p < 0.05).

**Effect of miR-10a on apoptosis of HK-2 Cells and expression of PI3K/AKT Signaling**

**Pathway Related Proteins**

The apoptosis rate and the expression of apoptosis-related proteins Bax and Caspase-3 were greatly increased and the expression of Bcl-2, PIK3CA, p-PI3K/PI3K, p-AKT/AKT proteins were notably decreased (p < 0.05) in the H/R group compared to the Control group (Figure 4). The apoptosis rate and the expression of apoptosis-related proteins Bax and Caspase-3 were remarkably increased and the expression of Bcl-2, PIK3CA, p-PI3K/PI3K, p-AKT/AKT proteins were greatly decreased in the miR-10a group than the H/R group (p < 0.05). Oppositely, the above indicators were outstandingly improved in the anti-miR group versus the H/R group (p < 0.05). Similarly, the above indicators were outstandingly improved in the anti-miR group in constrast to miR-10a group (p < 0.05).

**Dual luciferase report**

Bioinformatics search determined that PIK3CA was a target of miR-10a (Figure 5). To further verify whether miR-10a targeted PIK3CA, a dual luciferase reporter system was used. The results showed that miR-10a reduced the luciferase activity of PIK3CA containing the WT 3'UTR, but did not decrease the luciferase activity of PIK3CA containing the Mut 3' UTR.

**Effect of miR-10a targeting PIK3CA on cell proliferation**

Compared with the Control group, the level of PIK3CA mRNA was obviously down-regulated in the other groups showed in Figure 6, and the cell proliferation activity was greatly decreased (p < 0.05). The level of PIK3CA mRNA was remarkably down-regulated in the miR-10a in contrast to H/R group, si-P and miR + P-NC groups, and the cell proliferation ability was notably decreased (p < 0.05). The expression of PIK3CA mRNA was significantly increased in the miR + P group, and the cell proliferation ability was significantly increased versus the miR-10a group (p < 0.05).

**Effect of miR-10a targeting PIK3CA on apoptosis and expression of PI3K/AKT signaling pathway-related proteins**

As seen in Figure 7, the apoptosis rate of the other groups and the expression of apoptosis-related
proteins Bax and Caspase-3 were significantly increased than the Control group, and the expression of Bcl-2, PIK3CA, p-PI3K/PI3K, p-AKT/AKT were greatly decreased (p < 0.05). Also, the apoptosis rate and Bax and Caspase-3 were significantly increased in miR-10a in comparison with H/R group, si-P and miR + P-NC groups and the expression of PIK3CA, p-PI3K/PI3K, and p-AKT/AKT were greatly decreased (p < 0.05). When comparing with miR-10a group, the apoptosis rate and Bax and Caspase-3 were obviously decreased in miR + P group and the expression of Bcl-2, PIK3CA, p-PI3K/PI3K, p-AKT/AKT were significantly increased (p < 0.05).

Discussion
At present, Renal IRI is known as a primary reason of acute kidney failure and renal damage [21, 22]. However, advances in clinical treatments and progressive perspectives into the underlying mechanisms for effective treatments in alleviating IRI are still urgent [23]. The present work that performed on in vivo and in vitro studies illuminated the effect of miR-10a on renal IRI. And the results proved that miR-10a could alleviate ischemia-reperfusion-induced renal damage by targeting PIK3CA and inhibit the activity of PI3K/AKT signaling pathway.

miRNA has been attracted more and more wide attention and the results of continuing progress of the related research suggest that miRNA plays a crucial role in different human physiological and pathological course, and its biological activity is becoming increasingly clear, especially in cancer [24]. For miR-10a, it interacts with the 5’ untranslated region of mRNAs encoding RPs (ribosomal proteins) to promote their translation. In addition, miR-10a was discovered up-regulated in colon cancer, glioblastoma, hepatocellular carcinomas, and acute myeloid leukemia with NPM1 mutations [25, 26]. However, beyond that miR-10a is also widely expressed in adult mice and expression was highest in the kidney in kidney, lung and muscle. miR-10a as well as miR-10b expression is up-regulated when the tissue is injured [27]. In this research, it was demonstrated that renal tissue miR-10a gene was remarkably up-regulated in I/R group. And then the role and potential mechanisms of miR-10a on renal function and apoptosis were explored. The results showed that the serum Cr, BUN levels, renal injury score and apoptosis index were significantly increased. The expression level of Bcl-
2 was reduced and the Bax, Caspase3 were markedly increased. All the results demonstrated that miR-10a can promote the injury and apoptosis of the renal tissues.

The PI3K/Akt signaling pathway was primitively considered to have great effect in regulating the progress of cells proliferation and survival, which present has been proved to be relevant to the defending of brain, myocardium, lung, liver, and kidney against I/R injury by decreasing oxidative stress and inflammatory reaction [28-31]. lately it has been proved that the upregulation of the phosphorylation of Akt has great hepatoprotective effect on rat liver [32-34]. These findings indicated that Akt signaling pathway maybe an underlying cure target of different diseases [35-37]. Moreover, the phosphorylation of Akt was increased in renal tissues by I/R [38]. And the research noted that PI3K/Akt pathway, at least partly, was relevant to the protective effects on renal I/R injury. However, the upstream regulation mechanism of PI3K/Akt was not clarified. In present study, the dual luciferase assay was used to verify that PIK3CA was a target gene of miR-10a, and miR-10a can target PIK3CA and inhibit PI3K/AKT signaling pathway and then aggravate ischemia-reperfusion-induced renal injury.

Conclusions
To sum up, the present work explored the effect of miR-10a on renal IRI. Importantly, the results indicated that miR-10a can aggravate ischemia-reperfusion-induced renal injury by targeting PIK3CA and inhibit PI3K/AKT signaling pathway. Meanwhile, the results provide new insight into the interaction of miR-10a and ischemia-reperfusion-induced renal injury, which could be a novel anabolic therapy for renal injury disease.

Abbreviations
IRI: ischemia-reperfusion injury; 3’UTRs: 3’ un-translated region; PI3K: phosphatidylinositol 3-kinase;
AI: Apoptotic index

Declarations
Ethics approval and consent to participate
All animal experiments were conducted in accordance with the NIH guidelines (NIH Pub. No. 85-23, revised 1996) and have been reviewed and approved by the Animal Protection and Use Committee of the University of Qingdao Fuwai Cardiovascular Hospital.

Consent for publication
All co-authors have seen and agree to the manuscript for publication. We certify that the submission is original work and is not under review at any other publication.

**Competing interests**

The authors declare that they have no competing interests, and all authors confirm its accuracy.

**Funding**

No.

**Author Contributions**

DS Xu carried out the experimental work and the data collection and interpretation. WJ Li participated in the design and coordination of experimental work, and acquisition of data. DS Xu and G Wang carried out the study design, the analysis and interpretation of data and drafted the manuscript. T Zhang participated in the study design, data collection, analysis of data and preparation of the manuscript. All authors read and approved the final manuscript.

**Acknowledgments**

None.

**Availability of data and materials**

The figures data used to support the findings of this study were supplied by T Zhang under license and so cannot be made freely available. Requests for access to these data should be made to T Zhang, bgpzopzk@163.com.

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Figures
Effect of miR-10a on renal function in rats. (A) RT-PCR detection of miR-10a expression in kidney tissue; (B) expression of miR-10a gene in renal tissue of each group (C) determination of serum Cr content; (D) determination of serum BUN content; (E) HE staining to observe pathological changes in renal tissue; (F) Damage Score. *p < 0.05 compared to the Sham group; #p < 0.05 compared to the I/R group; ^p < 0.05 compared to the miR-10a group.
Effect of miR-10a on renal tissue apoptosis and expression of PI3K/AKT signaling pathway-related proteins in rats. (A) TUNEL staining was used to observe the apoptosis of renal cells; (B) Western blot was used to detect the expression of apoptosis-related proteins in renal tissues; (C) Western blot was used to detect the expression of PIK3CA and PI3K/AKT signaling pathway-related proteins in renal tissues. *p < 0.05 compared to the Sham group; #p < 0.05 compared to the I/R group; ^p < 0.05 compared to the miR-10a group.
Figure 3

Effect of miR-10a on HK-2 cell proliferation. (A) RT-PCR was used to detect the expression of miR-10a in cells; (B) CCK8 was used to detect the proliferative capacity of cells after transfection; (C) Cloning formation experiments. ap < 0.05 compared with the Control group; bp < 0.05 compared to the H/R group; cp < 0.05 compared to the miR-10a group.
Figure 4

Effect of miR-10a on apoptosis and expression of PI3K/AKT signaling pathway-related proteins in HK-2 cells. (A) Apoptosis rate was detected by flow cytometry; (B) Western blot was used to detect the expression of apoptosis-related proteins; (C) Western blot was used to detect the expression of PIK3CA and PI3K/AKT signaling pathway-related proteins. ap < 0.05 compared with the Control group; bp < 0.05 compared to the H/R group; cp < 0.05 compared to the miR-10a group.
Dual luciferase report. (A) Binding site predicted by miR-10a and PIK3CA region; (B) Double luciferase reporter result of recombinant vector of miR-10a and targeted gene PIK3CA. *p < 0.05* compared to the miR-NC group.
Effect of miR-10a targeting PIK3CA on cell proliferation. (A) RT-PCR was used to detect the expression of PIK3CA mRNA in cells; (B) CCK8 was used to detect the proliferative capacity of cells; (C) Cloning formation experiments. ap < 0.05 compared with the Control group; bp < 0.05 compared to the H/R group; cp < 0.05 compared to the miR-10a group.
Effect of miR-10a targeting PIK3CA on apoptosis and expression of PI3K/AKT signaling pathway-related proteins. (A) Apoptosis rate was detected by flow cytometry; (B) Western blot was used to detect the expression of apoptosis-related proteins; (C) Western blot was used to detect the expression of PIK3CA and PI3K/AKT signaling pathway-related proteins. 
ap < 0.05 compared with the Control group; bp < 0.05 compared to the H/R group; cp < 0.05 compared to the miR-10a group.

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