NLRP2 is highly expressed and promotes apoptosis in a mouse model of kidney ischemia/reperfusion injury

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Abstract
The aim of this study was to investigate the role of nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing 2 (NLRP2) in kidney ischemia/reperfusion injury. A mouse model of acute kidney ischemia/reperfusion injury was established to conduct in vivo experiments. Oxygen–glucose deprivation (OGD) and cobalt chloride treatment of the HK-2 and glomerular endothelial cell (GENC) kidney cell lines were performed for the in vitro study. Reverse transcription–quantitative polymerase chain reaction, western blotting, and immunohistochemical staining were used to analyze NLRP2 expression levels. Knockdown of NLRP2 in cells was also performed, and cell apoptosis was detected using flow cytometry. NLRP2 was expressed in normal kidney tissues; however, its expression was significantly increased in the acute kidney injury model and in OGD-treated cells. Conversely, knockdown of NLRP2 reduced apoptosis of cells. These results suggested that NLRP2 was involved in kidney damage and may be an important target for treatment of acute kidney injury.

Keywords
apoptosis, inflammasome, ischemia, kidney injury, NLRP2

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Introduction
It is well known that antigens and acute damage signals bind to extracellular or intracellular pattern recognition receptors (PRRs) to activate the innate immune response.1 Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are a family of intracellular PRRs, which share a common domain architecture, including a variable N-terminal effector domain, a NACHT domain, and a C-terminal leucine-rich repeat (LRR) domain. A total of 14 of the 22 human NLRs belong to the NLRP family (NLRP1-NLRP14).2-3 NLRP2 was demonstrated to act as a scaffold for caspase-1 activation, which leads to the activation and subsequent release of the key early response cytokine IL-1β.4 Moreover, a previous research has reported that NLRP2 contributes to the amplification of inflammatory responses by inhibiting the nuclear factor (NF)-κB pathway.5 In addition, Cheon et al.6 argued that the levels of NLRP2, IL-1β, and IL-18 were increased in brain tissues of a mouse model of ischemic stroke. Interestingly, existing evidence has revealed that NLRP3 initiated downstream inflammatory responses via interacting with apoptosis-associated speck-like protein (ASC).7 Mariathasan8 noted that the co-expression

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of NLRP3 and ASC could stimulate caspase-1 into activation to trigger IL-1β immune effects. It is reported that NLRP2 can counteract the interaction between NLRP3 and ASC by ASC binding to NLRP2-N-terminal domains.9

Kidney ischemia/reperfusion injury (KIRI) is a frequent renal disease, which was usually accompanied by tubular cell death triggered by tubular necrosis and apoptosis.10 Currently, many researchers have examined the NLRP3 expression variation in several kidney diseases. Kim et al.11 found that NLRP3 was located in the cytoplasm of proximal tubular cells and its expression level was raised during hypoxia. A recent study demonstrated that the inhibition of NLRP3 expression might be closely associated with acute kidney injury induced by lipopolysaccharide.12 However, the influence of NLRP2 on renal disease development, especially for KIRI, has not been elucidated. In this study, the functions of NLRP2 in the kidney in both steady-state and acute kidney injury were examined.

Materials and methods

Mouse model of acute kidney injury

All mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Animals were kept on a 12-h light/dark cycle at 18°C–22°C, 50%–70% humidity, and fed ad libitum unless stated otherwise. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A total of 24 8-week-old male C57/BL6J mice (weighing 22 g) were used for the acute kidney injury model. Renal ischemia/reperfusion (I/R) injury was performed to establish the acute kidney injury model, as previously described.13–15 Briefly, mice were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital. Subsequently, the mouse enterocoelia was exposed by a midline abdominal incision and bilateral renal pedicles were clamped for 30 min using microaneurysm clamps. Following removal of the clamps, reperfusion of the kidneys was visually confirmed. The incidence was then closed and the animal was allowed to recover. During ischemia, the body temperature was maintained between 36°C and 37.5°C using a temperature-controlled heating system. The same operation was performed on mice in the sham group, except for clamping of the renal pedicles. The mice were placed in their original cages for 12, 24, or 48 h following induction of ischemia (n = 6 per group), before being sacrificed. Mice in sham group were sacrificed together with the 24-h group. Subsequently, blood was collected from the ventriculus sinister, the mice were perfused with normal saline solution for 1 min, and kidneys were harvested. This study was approved by the Ethics Committee on Animal Experiments of Medical School of Shandong University (no. LL-201702001, Jinan, China).

Creatinine and blood urea nitrogen measurement

Mouse blood was collected by retro-orbital puncturing or ventriculus sinister, and serum was separated by centrifugation (2000 × g, 5 min, 4°C). Subsequently, creatinine and blood urea nitrogen (BUN) levels in serum (by retro-orbital puncturing, without anesthesia) were determined using a cobas c 702 module (Roche Diagnostics GmbH, Mannheim, Germany).

Western blot analysis

Kidney tissues and cultured cells were homogenized or lysed in radioimmunoprecipitation assay buffer (1% NP-40, 0.1% SDS, 100 μg/mL phenylmethane sulfonl fluoride, 1% protease inhibitor cocktail, and 1% phosphatase I and II inhibitor cocktail) on ice and the supernatants were collected. Protein concentration was determined by bicinchoninic acid assay. A total of 15 μg of protein from each sample was mixed with loading buffer and loaded onto a 10% Tris-HCl gel for polyacrylamide gel electrophoresis. Proteins on the gel were transferred to a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA). The membrane was blocked with 5% bovine serum albumin (cat. no. V900933; Sigma–Aldrich; Merck KGaA, Darmstadt, Germany) in Tris-buffered saline containing 0.1% Tween 20 at room temperature for 1 h and incubated with primary antibodies at 4°C overnight. The primary antibodies used were as follows: rabbit anti-NLRP2 antibody (1:1000; cat. no. 15182-1-AP; ProteinTech Group, Inc., Chicago, IL, USA), rabbit anti-caspase-3 antibody (1:1000; cat. no. 19677-1-AP; ProteinTech Group, Inc.), and mouse anti-actin (1:5000; cat. no. 60008-1-Ig; ProteinTech Group, Inc.). Subsequently, the membrane was washed in Tris-buffered saline containing 0.1%
Tween 20 and incubated with secondary antibodies at room temperature for 1 h. The secondary antibodies used were as follows: horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (1:5000; cat. no. SA00001-2; ProteinTech Group, Inc.) and HRP-conjugated goat anti-mouse IgG antibody (1:5000; cat. no. SA00001-1; ProteinTech Group, Inc.). The proteins were detected using enhanced chemiluminescence substrate (cat. no. CW0048; CoWin Biosciences Co., Ltd., Shanghai, China) and the membrane was scanned using the ChemiDoc™ XRS system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Image Lab software version 6.0 (Bio-Rad Laboratories, Inc.) was used for densitometry.

**Histology, immunohistochemical staining, and immunofluorescent staining**

Mouse kidneys were embedded in 4% paraffin at room temperature for 24 h and cut into 4-μm-thick sections. Subsequently, the kidney sections were stained with hematoxylin and eosin (H&E), according to standard protocol.13

For immunohistochemical staining, kidney sections were deparaffinized in xylene for 15 min and rehydrated using a series of decreasing ethanol concentrations. Antigen retrieval was performed with sodium citrate buffer at 98°C for 30 min and sections were blocked with 5% bovine serum albumin (Sigma–Aldrich; Merck KGaA) at room temperature for 1 h, then incubated with primary rabbit anti-NLRP2 antibody (1:100; cat. no. 15182-1-AP; ProteinTech Group, Inc.) overnight at 4°C. After washing three times with phosphate-buffered saline (PBS), the kidney sections were incubated with goat anti-rabbit HRP-conjugated secondary antibody (1:500; cat. no. SA00001-2; ProteinTech Group, Inc.) at room temperature for 1 h and stained with 3,3′-diaminobenzidine (DAB; Sangon Biotech Co., Ltd., Shanghai, China).

For immunofluorescent staining, kidney sections were processed in the same way as for immunohistochemical staining and then incubated with primary antibodies overnight at 4°C. The primary antibodies used were as follows: rabbit anti-NLRP2 antibody (1:100; cat. no. 15182-1-AP; ProteinTech Group, Inc.), rabbit anti-aquaporin (AQP) 1 antibody (1:100; cat. no. sc-20810; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit anti-AQP3 antibody (1:100; cat. no. sc-20811; Santa Cruz Biotechnology, Inc.), and mouse anti-calbindin D28K antibody (1:100; cat. no. sc-135666; Santa Cruz Biotechnology, Inc.). Subsequently, the sections were incubated with secondary antibodies for 1 h at room temperature. The secondary antibodies used were as follows: donkey anti-rabbit IgG Alexa Fluor® 488 (1:1000; cat. no. ab150073; Abcam, Cambridge, UK), donkey anti-mouse IgG Alexa Fluor 594 (1:1000; cat. no. ab150108; Abcam), and donkey anti-rabbit IgG Alexa Fluor 594 (1:1000; cat. no. ab150080; Abcam).

The number of tubules in the corticomedullary junction that displayed cellular necrosis and loss of brush border were counted and scored in a blinded manner as follows: 0, none; 1, 0%–10%; 2, 11%–25%; 3, 26%–45%; 4, 46%–75%; and 5, >75%. At least 10 high-power fields (×200 magnification) per section for each sample were examined. All images were acquired with an Eclipse TE2000-U microscope (Nikon Corporation, Tokyo, Japan) using an HQ2 cooled CCD camera and a ×10 or ×20 objective lens.

**Cell culture, cobalt chloride (CoCl2), and oxygen–glucose deprivation treatment**

The human podocytes (HPC; cat. no. BNCC340460; BNCC, Kunshan, China), glomerular endothelial cell (GENC; cat. no. 4000; ScienCell Research Laboratories, Inc., San Diego, CA, USA), renal mesangial cell (RMC) SV40 MES 13, and immortalized human renal proximal tubule HK-2 cell lines were all purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C in a 5% CO2 environment, as previously described.16 CoCl2 was purchased from Sigma–Aldrich (cat. no. 60818; Merck KGaA) and the vehicle was water. When the cells reached ~80% confluence, CoCl2 was added to the cell culture medium at a final concentration of 125, 250, or 500 μM for 12 h.

To induce oxygen–glucose deprivation (OGD) conditions, the medium was replaced with glucose-free Earle’s balanced salt solution (cat. no. CM90113; Macgene Biotechnology Ltd., Beijing, China). Cells were transferred to an anaerobic chamber to ensure that the concentration of oxygen...
in the media <1%. A Tri-gas incubator (Thermo Fisher Scientific, Inc.) was used to limit the oxygen concentration to 0.5% and simulate a hypoxic environment. The chamber was then sealed and placed in the incubator at 37°C over a time course of 12, 24, and 48 h. For the control group, cells were kept under normoxic conditions (21% oxygen).

Small interfering RNA transfection

Cells were plated in 6-well plates (3 × 10^5 cells/well) and transfected with 75 nM NLRP2-siRNA (5′-TGT TTG ATG TCC GGT GAC ATC-3′; Guangzhou RiboBio Co., Ltd., Guangzhou, China) or negative control small interfering RNA (siRNA; Guangzhou RiboBio Co., Ltd.) using DharmaconFECT 1 Transfection Reagent (cat. no. T-2001-03; GE Healthcare Dharmacon, Inc., Lafayette, CO, USA). Cells were incubated at 37°C for 48–72 h before collection for subsequent experimentation, according to the manufacturer’s guidelines.

Detection of apoptotic cells

Cells were fixed in 4% paraformaldehyde at room temperature for 10 min. Apoptotic cell death was determined using terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) Apoptosis Assay Kit (Sangon Biotech Co., Ltd.). The nucleus was stained using 4′,6-diamidino-2-phenylindole (DAPI) at room temperature for 5 min. Mounting medium was purchased from Boster Biological Technology (cat. no. AR1109; Pleasanton, CA, USA). A total of eight fields of view per slide were observed.

Reverse transcription–quantitative polymerase chain reaction

RNA isolation and reverse transcription–quantitative polymerase chain reaction (RT-qPCR) were carried out as previously described. Briefly, total RNA was isolated from kidney tissue or cells using TRIzol® reagent (Thermo Fisher Scientific, Inc.). First-strand cDNA was synthesized using a reverse transcription system kit, according to the manufacturer’s protocol (CoWin Biosciences Co., Ltd., Taizhou, China). RT-qPCR was performed on the Roche LightCycler 480 Real-Time PCR system using SYBRGreen (cat. no. CW0955; CoWin Biosciences Co., Ltd.). The PCR cycling conditions were as follows: 95°C for 10 min, followed by 45 cycles of 95°C for 30 s, 60°C for 20 s, and 72°C for 15 s. The sequences of the primer pairs were as follows: mouse NLRP2—forward, 5′-AAC ACT GAG CCT GAA ACA CTT GGA-3′ and reverse, 5′-CAG TTC AGT GGA GTG ATG GAG CA-3′; mouse GAPDH—forward, 5′-TGT CTC CTG CGA CTG CAA TA-3′ and reverse, 5′-GGT CAG GTT CGG TTT CAT ACC-3′; human NLRP2—forward, 5′-CCG ACA ATG AGC TTC TGG ATG-3′ and reverse, 5′-AGC AAG GTC CTT GC-3′ and reverse, 5′-TCC ACC ACC CTG TTG CTG TA-3′. PCR was performed under standard conditions. The relative gene expression was calculated after normalizing to GAPDH, according to the 2^−ΔΔCq method.

Flow cytometry

Cell apoptosis was measured by flow cytometry. Briefly, ~1 × 10^6 cells were collected by centrifugation (200 × g, 4°C, 8 min) and washed with PBS. Subsequently, the cells were resuspended in binding buffer supplemented with 5 mL AnnexinV-phycoerythrin and 10 mL propidium iodide (50 mg/mL) and incubated at room temperature for 15 min in the dark (cat. no. KGA1017; Nanjing KeyGen Biotech Co., Ltd., Nanjing, China). The cells were acquired on a flow cytometer (Beckman Coulter, Inc., Brea, CA, USA) and the data were analyzed using CytExpert software version 1.0 (Beckman Coulter, Inc.).

Statistical analyses

All data were expressed as the mean ± standard error of the mean. Three independent experiments were performed. Statistical analysis of the data was performed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA).Comparisons between two groups were made using Student’s t-test, whereas comparisons between two or more groups were made using one-way analysis of variance followed by the least significance difference post hoc test. The value of P less than 0.05 was considered to indicate a statistically significant difference.

Results

Successful establishment of mouse renal I/R model of acute kidney injury

I/R injury is a commonly used model for study of acute kidney injury. In this study, I/R injury was used as the in vivo model for acute kidney...
injury. Sham and I/R surgery were performed, and following 24 h, the mice were sacrificed and the kidneys were harvested for H&E staining (Figure 1(a)). The results demonstrated that the tubular damage score and the serum levels of creatinine and BUN of the I/R group were significantly increased compared with the sham group, which indicated that kidney injury was successfully induced (Figure 1(b)). In addition to H&E staining, TUNEL staining was performed to investigate apoptosis in the tissue (Figure 1(c)). The results revealed that the apoptosis ratio (TUNEL-positive cells/total cells) of the I/R group was increased compared with the sham group (Figure 1(d)). Western blot analysis revealed that cleaved caspase-3 was significantly increased in the kidneys of mice in the I/R injury group (Figure 1(e) and (f)). Altogether, these data demonstrated that the I/R injury model for acute kidney injury was successfully established.

**NLRP2 expression is significantly increased in the kidneys of mice with I/R injury**

Mice were randomly assigned to one of four experimental groups: sham group, 12, 24, and 48 h after induction of I/R groups. Mice in the sham group were sacrificed together with the 24-h group. The mRNA expression levels of NLRP2 in the kidney tissues were detected. NLRP2 expression levels were highest in the 24-h group and then decreased in the 48-h group (Figure 2(a)), suggesting that NLRP2 transcription increased during acute kidney injury. In addition, western blot analysis was used to confirm whether protein levels of NLRP2 changed during I/R injury (Figure 2(b)). The results demonstrated that NLRP2 protein levels were significantly increased in the 12-h group. NLRP2 protein levels were highest in the 24-h group and these levels were almost sustained in the 48-h group (Figure 2(c)). One plausible explanation for expression discrepancy between levels of NLRP2 mRNA and protein was complicated regulation mechanisms such as post-translational modification and transcript degradation, which largely affected the abundance of these two molecules. Another possibility was that cross-hybridization effects of different mRNA molecule may directly affect the accuracy of hybridization-based mRNA quantitation but do not obscure protein measurements. Nevertheless, our findings clearly demonstrated that the expressions of NLRP2 mRNA and protein were significantly elevated within initial 24 h after acute kidney injury. Immunohistochemistry staining of NLRP2 in kidney paraffin sections was also performed to observe protein localization and abundance (Figure 2(d)). NLRP2 was increased in the I/R group and was mainly located at the renal tubules. This increase in NLRP2 strongly suggested that NLRP2 may be involved in acute kidney injury.

**NLRP2 is primarily expressed in renal tubules of an in vivo acute kidney injury model**

Using immunohistochemistry, it was identified that NLRP2 was primarily expressed in the renal tubules of the kidneys after I/R injury. However, to further define the tubular segment specificity of NLRP2 expression, double immunofluorescent staining for NLRP2 (green) and various tubular markers (red) was performed in kidney sections. The following segment-specific tubular markers were used: proximal tubule, AQP1; distal tubule, calbindin D28k; and collecting duct, AQP3. As presented in Figure 2(e)–(g), NLRP2 was mainly expressed in proximal and distal tubules, whereas lower expression was observed in the collecting duct (Figure 2(g)), indicating the potential tissue- or cell-specific expression patterns under stress conditions.

**NLRP2 is expressed in kidney-derived cell lines**

Next, the NLRP2 expression levels in kidney-derived cell lines were detected. The following cell lines were used: HPC, GENC, proximal tubule epithelial cells (HK-2), and RMCs. Western blot analysis was performed, and the results demonstrated that the highest NLRP2 protein levels were detected in the proximal tubule epithelial HK-2 cell line (Figure 3(a)). This was consistent with the previous results obtained from kidney tissue immunofluorescent staining, which indicated that the main injury occurred in the renal tubules of kidneys with acute kidney injury (Figure 2(e)–(g)).

**NLRP2 expression is markedly increased in HK-2 cells following OGD treatment**

I/R injury typically involves an immediate deprivation of both glucose and oxygen, which are needed to maintain the metabolic demands of the renal tubules. Thus, OGD is an appropriate in vitro
Figure 1. Establishment of the mouse renal I/R model for acute kidney injury. (a) Representative photomicrographs of hematoxylin and eosin stained kidney tissue sections from mice in the sham or I/R injury 24 h group. Scale bar, 50 μm. (b) Quantitative assessment of tubular damage. Serum levels of creatinine and blood urea nitrogen. (c) Detection of renal cell apoptosis by TUNEL assay. Nuclei were stained with DAPI. Scale Bar, 50 μm. (d) Percentage of TUNEL-positive cells from total cells per field. (e and f) Western blot analysis of cleaved caspase-3 in kidneys of mouse from the sham or I/R injury 24 h group. Student’s t-test; **p < 0.01, ***p < 0.001 vs sham group. I/R: ischemia reperfusion; TUNEL: terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling.
Figure 2. NLRP2 is expressed at basal levels in mouse kidney tissue and increases after I/R injury, with predominant expression in the renal tubules. (a) Relative mRNA levels of NLRP2 in the kidneys after sham or I/R operation. (b and c) Western blot analysis of NLRP2 expression in the kidneys after sham or renal I/R operation. (d) NLRP2 immunohistochemical staining of kidney tissue sections from mice in the sham or I/R 24 h group. Scale bar, 50 μm. (e–g) Co-immunofluorescence staining with NLRP2 and tubular segment-specific markers (proximal tubule, AQP1; distal tubule, calbindin D28k; and collecting duct, AQP3) of kidney sections from mice in the I/R 24 h group. Dotted box indicates regions shown at a higher magnification in the lower panel. Scale bar, 50 μm. One-way analysis of variance; *P < 0.05, **P < 0.01 vs sham group. AQP: aquaporin; I/R: ischemia reperfusion; NLRP2: nucleotide-binding oligomerization domain-like receptor pyrin domain containing 2.
model for I/R injury. HK-2 cells received OGD treatment for 12, 24, and 48 h, and subsequently, NLRP2 mRNA expression levels were detected. The result revealed that NLRP2 mRNA expression levels were significantly increased and peaked following 24 h OGD treatment (Figure 3(b)), which was consistent with the in vivo I/R experiment results (Figure 2(a)). Next, the protein expression levels of NLRP2 in HK-2 cells after OGD treatment were examined (Figure 3(c)). The results demonstrated that NLRP2 protein levels increased significantly and reached a peak following 24 h OGD treatment (Figure 3(d)), which was again consistent with the in vivo I/R experiment results (Figure 2(b)). To further confirm the in vitro hypoxia injury results, hypoxia conditions were mimicked by treating HK-2 cells with CoCl₂. HK-2 cells were exposed to vehicle or different concentrations of CoCl₂ (125, 250, and 500 μM), prior to western blot analysis of NLRP2 (Figure 3(e)). The result revealed that CoCl₂ significantly increased NLRP2 protein levels and the peak was reached at 250 μM (Figure 3(f)). The in vitro and in vivo data collectively demonstrated that NLRP2 expression was affected during acute kidney injury and strongly suggested it may serve an important role during this process.

Silencing of NLRP2 reduces OGD-induced cell apoptosis of HK-2 cells

NLRP2 protein levels were increased both during I/R injury in vivo and OGD treatment in vitro; therefore, it was hypothesized that NLRP2 may be involved in apoptosis. In order to assess the effect of NLRP2, NLRP2 was knocked down. HK-2 cells were transfected with NLRP2 siRNA (siNLRP2) or the control RNA (siCON). The protein expression levels of NLRP2 were measured to confirm knockdown efficiency. NLRP2 protein levels were
0.2-fold the expression of the control group, which suggested that the knockdown efficiency was high. Subsequently, flow cytometry was performed to assess apoptosis. The results revealed that under normal oxygen and glucose conditions, knockdown of NLRP2 did not alter the proportion of apoptotic HK-2 cells (92.3%–92.1%), while under OGD conditions, knockdown of NLRP2 significantly reduced the number of apoptotic cells (69.5%–80.6%). In addition, cleaved caspase-3 was detected by western blotting. OGD treatment significantly increased cleaved caspase-3 levels when cells expressed NLRP2, but when NLRP2 was knocked down, the levels of cleaved caspase-3 were significantly decreased. These results strongly suggested that NLRP2 participated in I/R-induced acute kidney injury and may aggravate the damage caused by ischemia.

**Discussion**

NLRs are intracellular PRRs, which have crucial roles in the innate immune response by recognizing damage-associated molecular patterns and pathogen-associated molecular patterns. A few members have been extensively studied, including NLRP1, NLRP3, and NLRP6, which can recognize a wide range of endogenous and exogenous damage-associated molecular patterns. This leads to activation of caspase-1, processing and release of IL-1β and IL-18, and results in inflammation. NLRP2 is less studied, especially in the context of kidney diseases, including acute kidney injury. NLRP2 has been revealed to be expressed in many organs, including the kidney, brain, and testis. It has also been reported that NLRP2 is associated with the inflammatory response.

Acute kidney injury is a serious clinical complication with high morbidity and mortality rates, and the role of NLRP2 in this process is largely unknown. In this study, experiments were conducted to explore the role of NLRP2 in acute kidney injury. The expression levels of NLRP2 at both the transcriptional and translational levels were measured, and it was demonstrated that NLRP2 expression in kidney tissues was increased during I/R injury. To the best of our knowledge, this is the first study to report on the role of NLRP2 in acute kidney injury. Subsequently, the localization of NLRP2 during renal I/R injury was investigated and it was revealed that it was mostly located in the renal tubule segment, which is a novel finding. In order to further confirm this observation, the expression of NLRP2 in different kidney-derived cell lines was measured and it was identified that NLRP2 was most highly expressed in tubule cells, which was consistent with results obtained using kidney tissues. Finally, siRNA was utilized to knock down NLRP2 and the results demonstrated that a reduction in NLRP2 expression could reduce OGD-induced cell apoptosis, suggesting that NLRP2 mediates inflammatory responses that may promote cell apoptosis during acute kidney injury.

In conclusion, the data demonstrated that NLRP2 increased following renal I/R injury, it was mostly expressed in the tubule segment of the kidneys, and could promote cell apoptosis during acute kidney injury. Therefore, targeting NLRP2 may represent a novel strategy for the treatment of acute kidney disease.

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**Author contributions**

X.Y. and Z.H. designed the experiments; X.Y. and X.Z. performed the experiments; and all the authors wrote the article.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the author X.Y. on reasonable request.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical approval and consent to participate**

The present study was approved by the Ethics Committee on Animal Experiments of Medical School of Shandong University (no. LL-201702001 Jinan, China).

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