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# Effects of homeodomain-interacting protein kinase 2 on renal tubular epithelial cells

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**Abstract. – OBJECTIVE: To research the role of homeodomain-interacting protein kinase 2 (HIPK2) on the oxidative stress (OS) and apoptosis induced by hypoxia/reoxygenation (H/R) of normal rat kidney-52E (NRK-52E) cells, through the JAK2/STAT3 signaling pathway**

**MATERIALS AND METHODS: First, we transfected the NRK-52E cells with small interfering RNA (siRNA) of HIPK2 by LipofectamineTM 2000, Real Time-Polymerase Chain Reaction (RT-PCR) and Western blot (WB) were used to test the efficiency of transfection after 48 h. After cells were treated with H/R, we tested cell apoptosis by Cell Counting Kit-8 (CCK-8) assay, flow cytometry, TUNEL staining, PCR, and Western blot.**

**RESULTS: After NRK-52E cells were transfected with siRNA-HIPK2, the protein expression of HIPK2 was remarkably decreased. Cell apoptosis and OS in the H/R group were significantly increased. However, in the HIPK2-siRNA + H/R group, apoptosis and OS were markedly decreased compared with the H/R group.**

**CONCLUSIONS: Inhibition of HIPK2 expression can promote H/R-induced proliferation of NRK-52E cells through the JAK2/STAT3 signaling pathway, relieve the OS response, and reduce apoptosis.**

*Key Words:*
Homeodomain-interacting protein kinase 2 (HIPK2), Oxidative stress (OS), Apoptosis, NRK-52E, JAK2/STAT3 signaling pathway.

## Abbreviations

Acute kidney injury: AKI; chronic kidney disease: CKD; end-stage renal disease: ESRD; ischemia reperfusion injury: IRI; Renal tubular epithelial cells: RTEC; endoplasmic reticulum stress: ERS; extracellular matrix: ECM; glomerular epithelial cell: GEC; mitochondrial permeability transition pore: MPTP; Homeodomain-interacting protein kinase: 2 HIPK2; hypoxia/reoxygenation: H/R; Oxidative stress: OS; Malondialdehyde: MDA; Superoxide Dismutase: SOD; Glutathione Peroxidase: GSH-Px; Reactive Oxygen Species: ROS; 8-hydroxydeoxyguanosine: 8-OH-dG; 5-hydroxy deoxycytosine: 5-OH-dC; small interfering RNA: siRNA; Homeodomain Interacting Protein Kinases: HIPKs.

## Introduction

Acute kidney injury (AKI) is a common clinical critical illness with high morbidity and mortality, which has brought a heavy burden on society. In addition, severe AKI can cause chronic kidney disease (CKD) and eventually lead to end-stage renal disease (ESRD). AKI is currently considered one of the main risk factors for CKD, and ischemia reperfusion injury (IRI) is an important factor causing the disease[1]. In recent years, new techniques, such as catheterization, thrombolytic therapy, and kidney transplantation are often used in clinic to restore blood flow to ischemic renal tissue, but it has caused renal IRI[2]. Renal tubular epithelial cells (RTEC) is more sensitive to injury factors, such as sepsis, poisoning, and ischemia and hypoxia. Hypoxia injury is an important cause of various kidney diseases. The main lesions are apoptosis and necrosis of RTEC, and the proliferation, dedifferentiation, and redifferentiation of tubular epithelial cells are the basis for repair after injury[3]. Therefore, it is of great significance to find effective therapeutic targets for AKI.

HIPK2 is a member of the HIPK family. It is involved in regulating gene expression and is activated by signal induction[4]. Fan et al[5] have reported that HIPK2 is a key regulator in the process of renal fibrosis, and it can cause renal



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fibrosis when highly active. However, the role of HIPK2 in AKI is unclear. Therefore, in this study, small interfering RNA (siRNA) of HIPK2 was transfected into NRK-52E and treated with hypoxia/reoxygeneration (H/R). The changes of OS and apoptotic levels in NRK-52E cells were detected to explore the mechanism of action of HIPK2 on NRK-52E cells.

The JAK/STAT signaling pathway can regulate the expression of stress response genes, affect cell proliferation, differentiation, migration, and apoptosis, and play an important role in various pathophysiological processes, such as hematopoietic, immune response, inflammation, development, and growth[6]. Although the structure of the JAK protein family is highly correlated, the distribution of each protein, upstream activator, and downstream effector are highly specific. JAK can regulate the phosphorylation of members of the STAT family, thereby promoting the transfer of transcription factors into the nucleus and enhancing gene transcription[7]. STAT family protein exists in the mitochondria and regulates the adjustable mitochondrial respiratory regulation of cells, the apoptosis and mitochondria process, and the inhibition of the mitochondrial permeability transition pore (MPTP) open[8]. Although JAK/STAT signaling is most prominent in lymphocytes, it is also present in renal cells, such as podocytes, mesangial cells, and RTEC[9]. Thus, the JAK/STAT signaling pathway is closely related to the progression of kidney disease.

## Materials and Methods

### Cell Culture and Grouping

NRK-52E cells (Cell Culture Center, Shanghai, China) frozen in liquid nitrogen were taken out and rapidly thawed in a water bath at 37°C. Then, the cells were placed in an incubator containing 5% $CO_2$ and 95% $O_2$ and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) medium containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 U/mL penicillin (Gibco, Rockville, MD, USA), 100 μg/mL (Gibco, Rockville, MD, USA) streptomycin. We changed the medium regularly. After 80% of the cells were fused, they were replaced with high-sugar culture medium without FBS and incubated for 24 h. Then, NRK-52E cells were divided into control group, HIPK2-NC group, H/R group, and HIPK2-siRNA + H/R group.

### HIPK2-siRNA Transfected NRK-52E Cells

One day before transfection, the cells were inoculated in 12-well plates. The next day, we used Lipofectamine™ 2000 (Corning, Corning, NY, USA) to transfect cells. After 6 h, the cell culture medium was changed and the cell status was observed. Then, the cells were collected after 48 h.

### Cell Treatment

We took the cells after 48 h of transfection, changed the culture medium to phosphate-buffer solution (PBS) before hypoxia preparation, and cultured them in a 37°C incubator containing 1% $O_2$, 4% $CO_2$ and 95% $N_2$ mixed gas. After 1 h, we changed normal medium culture, and placed the cells in an incubator with 5% $CO_2$ and 95% $O_2$ for 2 h, and collected them. In addition, we added JAK/STAT specific inhibitor WP1066 (GLPBIO, Montclair, CA, USA) after the cells were reoxygenated for 2 h. WP1066 was dissolved with dimethyl sulfoxide (DMSO) reagent and stored at 4°C.

### Cell Counting Kit-8 (CCK8) Assay to Detect Cell Viability

After H/R treatment, CCK-8 method (Dojindo Molecular Technologies, Kumamoto, Japan) was used to detect cell viability in each group. According to the instructions of the CCK-8 kit, after adding the CCK-8 solution to the wells to be tested, incubation was continued for 2 h in the dark. The absorbance of each well was then measured at 490 nm to reflect cell viability and absorbance values.

### Cell Supernatant Detection of 8-OH-dG, 5-OH-dC Content

The cell supernatants of each group were collected. In order to detect the degree of DNA damage caused by OS, we used 8-OH-dG and 5-OH-dC kits (Jian Cheng, Nanjing, China) to detect the degree of damage. The experiment was repeated 3 times.

### Detection of Malondialdehyde (MDA) Content, and Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD) Activity

The cells of each group were treated according to the corresponding groupings mentioned above. The MDA content, SOD, and GSH-Px activity

in the culture medium of each group were measured, according to the instructions of the assay kit (Jian Cheng, Nanjing, China).

### TUNEL Staining

We removed the treated NRK-52E cells and permeated the cell membrane with Triton X-100 (Elabscience, Wuhan, China) according to the instructions of the TUNEL staining kit (Elabscience, Wuhan, China). Then, we stained the HRK-52E cells with TUNEL reagent. After 4',6-diamidino-2-phenylindole (DAPI) staining, the number of positive cells was observed under a fluorescent inverted microscope.

### Detection of Intracellular Reactive Oxygen Species (ROS) Levels

We collected the treated NRK-52E cells in the 6-well plate, processed the NRK-52E cells according to the instructions of the ROS kit (Elabscience, Wuhan, China), and then, sent the sample to the flow cytometry center to detect the ROS level. The experiment was repeated 3 times.

### Detection of Apoptosis by Flow Cytometry

We inoculated 5 x $10^5$ NRK-52E cells into a 6-well plate. The cells were collected after trypsinization, and then the cells were washed with PBS, twice. Then, we added the binding buffer 500 μL to resuspend the cells, added Annexin V-FITC (Thermo Fisher Scientific, Waltham, MA, USA) 5 μL, and incubated them in a dark environment at 4°C for 15 min. Then, we added Propidium Iodide (PI; Thermo Fisher Scientific, Waltham, MA, USA) 5 μL, and placed them in a dark environment for 5-15 minutes. Then, we sent the sample to the flow cytometry center to detect the apoptosis level. The experiment was repeated 3 times.

### $Ca^{2+}$ Fluorescence Detection

The cells were collected and washed twice with PBS, and 1 μmol/L calcium sensitive fluorescent probe Fluo-3/AM was added to the cells. We mix the cells thoroughly with shaking, and incubated them at 37°C in the dark for 45 minutes. Then, the cells were washed with PBS, and sent for inspection after 15 minutes of incubation. The emission and excitation wavelengths of Fluo-3/AM are 526 nm and 488 nm, respectively. The intracellular free $Ca^{2+}$ concentration was reflected by the fluorescence intensity, and the experiment was repeated 3 times.

### Western Blot

We took out the treated NRK-52E cells, and added cell lysate to extract the protein in the cells, and quantified the protein in the bicinchoninic acid (BCA) kit (Jian Cheng, Nanjing, China). Then, we took 40 μg of protein sample and loading buffer, mixed thoroughly and boiled them for 5 min at 100°C, and performed sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) separation. After the electrophoresis was completed, the protein was transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), then, it was blocked with 5% skim milk powder for 2 h, and the antibody was incubated at 4°C overnight. The next day, after washing the PVDF membrane, horse radish peroxidase (HRP)-labeled goat anti-rabbit antibody (Thermo Fisher Scientific, Waltham, MA, USA) was added and incubated for 2 h. Then, the enhanced chemiluminescence reagent (ECL; Thermo Fisher Scientific, Waltham, MA, USA) was added, and the membrane was exposed. The Quantity One software analyzes the protein gray value. The antibodies used in this experiment are as follows: p-JAk2, 1:1000, Abcam, Cambridge, MA, USA, Rabbit; p-STAT3, 1:1000, Abcam, Cambridge, MA, USA, Rabbit; Bax, Bcl-2, 1:1000, Abcam, Cambridge, MA, USA, Mouse; Caspase3, Caspase12, 1:1000, Abcam, Cambridge, MA, USA, Mouse; Ki67, 1:1000, Abcam, Cambridge, MA, USA, Rabbit; SOD1,-SOD2, 1:2000, Abcam, Cambridge, MA, USA, Rabbit; glyceraldheyde 3-phosphate dehydrogenase (GAPDH), Abcam, Cambridge, MA, USA, 1:2000, Abcam, Cambridge, MA, USA, Rabbit).

### Real Time-Polymerase Chain Reaction (PCR)

Total RNA was extracted according to the method of RNA extraction kit (Thermo Fisher Scientific, Waltham, MA, USA). We reversed the RNA to complementary deoxyribose nucleic acid (cDNA) according to the instructions of the reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, USA), and then, performed PCR amplification. We took 1 μL of the above-mentioned reverse transcription product as the reaction template, and performed the Real Time-PCR reaction. The reaction system was 10 μL. The primers were synthesized by Biotechnology Bioengineering (Shanghai, China), as shown in Table I. Real Time-PCR reaction conditions: 95°C, 4 min pre-denaturation; 95°C, 15 s denaturation, 60°C, 30 s annealing, for a total of 39

cycles. With GAPDH as the internal reference gene, the relative expression of the target gene was determined.

### Statistical Analysis

All experimental data were analyzed using Statistical Product and Service Solutions (SPSS) 21.0 software (IBM Corp., Armonk, NY, USA). The measurement data were expressed as mean ± standard deviation (mean ± SD). Comparison of multiple groups was performed by single factor analysis of variance. Pairwise comparisons between groups were performed using Bonferroni-corrected *t*-test. $p<0.05$ was considered statistically significant.

## Results

### Detection of HIPK2-siRNA Transfection Efficiency and its Effect on Cell Viability

First, we used Western blot and PCR methods to verify the transfection efficiency of NRK-52E cells. The results showed that HIPK2 protein expression was significantly lower in the HIPK-siRNA group (Figure 1A, $p<0.05$), while the expression of HIPK2 in the HIPK2-NC group was not dramatically different from that in the control group. This indicated that the transfection of HIPK2-siRNA can dramatically reduce the expression of HIPK2. Meanwhile, PCR also confirmed that the expression of HIPK2 in the HIPK2-siRNA group was dramatically reduced (Figure 1B, $p<0.05$). Then, we constructed the H/R model of NRK-52E cell, in order to detect whether interference with HIPK2 could alleviate the decreased cell activity caused by H/R. Through CCK-8 experiments, we found that compared with the control group, NRK-52 cells in the H/R group had a slightly increased vitality after reoxygenation (Figure 1C). When we intervened HIPK2, the HIPK2-siRNA + H/R group cells viability remarkably increased ($p<0.05$).

### Effect of HIPK2-siRNA Transfection on H/R Induced OS

As can we can see from Table II, the contents of serum 8-hydroxydeoxyguanosine (8-OH-dG) and 5-hydroxy deoxycytosine (5-OH-dC) in H/R group increased by 167.23% and 236.35% respectively ($p<0.05$), indicating that DNA oxidative damage occurred in NRK-52E cells in H/R group. However, the levels of 8-OH-dG and 5-OH-dC in the supernatant decreased by 32.31% and 49.87% respectively after HIPK2 was interfered ($p<0.05$). The above results showed that when we interfered HIPK2 with NRK-52E cell, the H/R induced DNA oxidative damage could be dramatically repaired. At the same time, the intracellular ROS level was also detected by flow cytometry, and the results showed that the ROS level of the H/R group was dramatically increased (Figure 2A, $p<0.05$), but the ROS level of the HIPK2-siRNA + H/R group was significantly decreased ($p<0.05$). Next, we tested the antioxidant enzyme activity and MDA content in the supernatant (Figure 2B-2D). In the H/R group, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were remarkably reduced ($p<0.05$), and malondialdheyde (MDA) content was significantly increased ($p<0.05$). By contrast, when we interfered with HIPK2, we found that SOD and GSH-Px activities were markedly increased ($p<0.05$), while MDA content was significantly inhibited ($p<0.05$). Secondly, we detected the expression of SOD1 and SOD2 by Western blot and PCR (Figure 2E and 2F), and found that the expression of SOD1 and SOD2 in the H/R group was reduced ($p<0.05$), while the expression of SOD1 and SOD2 was increased

**Table I.** Real Time-PCR primers.

| Gene name | Forward (5′>3′) | Reverse (5′>3′) |
|---|---|---|
| SOD1 | GGTGAACCAGTTGTGTTGTC | CCGTCCTTTCCAGCAGTC |
| SOD2 | CAGACCTGCCTTACGACTATGG | CTCGGTGGCGTTGAGATTGTT |
| Caspase-3 | ATGGAGAACAACAAAACCTCAGT | TTGCTCCCATGTATGGTCTTTAC |
| Bax | TGAAGACAGGGGCCTTTTTG | AATTCGCCGGAGACACTCG |
| Bcl-2 | GTCGCTACCGTCGTGACTTC | CAGACATGCACCTACCCAGC |
| Caspase-12 | TTGGATACTCAGTGGTGATAAAGGA | GGATGCCGTGGGACATAAAGA |
| Ki67 | AGTGTGTCCGGCTTCAAC | AGATTCCTCCTCAGCACCT |
| GAPDH | ACAACTTTGGTATCGTGGAAGG | GCCATCACGCCACAGTTTC |

qRT-PCR, quantitative Reverse-Transcription Polymerase Chain Reaction.

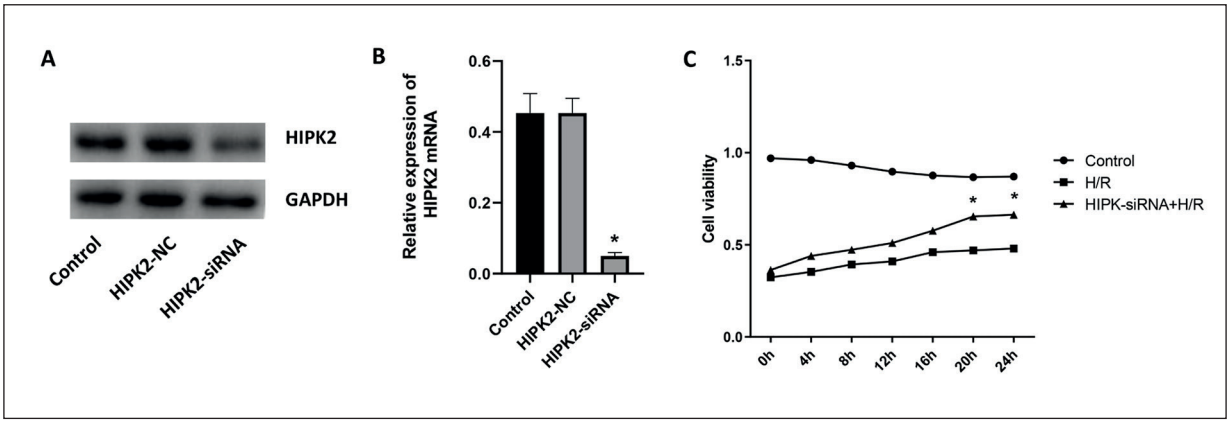


**Figure 1.** Detection of HIPK2-siRNA transfection efficiency and its effect on cell viability. **A,** WB detection of HIPK2 transfection efficiency. **B,** PCR detection of HIPK2 transfection efficiency. **C,** CCK8 detection of cell viability. Mean ± SD, n = 3, "*" $p<0.05$ *vs.* Control group.

when we interfered NRK-52E cells with HIPK2 ($p<0.05$).

### Effect of HIPK2-siRNA Transfection on H/R Induced Proliferation and Apoptosis

After we collected three groups of cells, using flow cytometry to detect cell apoptosis rate and $Ca^{2+}$ fluorescence intensity (Figure 3A and 3B), the results showed that H/R cell apoptosis rate was increased ($p<0.05$), while the HIPK2-siRNA + H/R group cell apoptosis rate was decreased ($p<0.05$). At the same time, H/R group of $Ca^{2+}$ fluorescence intensity value was significantly higher than the control group ($p<0.05$), while HIPK2-siRNA + H/R group of $Ca^{2+}$ fluorescence intensity values were below than the H/R group ($p<0.05$). We observed TUNEL staining with fluorescence microscope (Figure 3C). We found that after H/R treatment, the apoptotic rate of cells increased significantly ($p<0.05$), but the number of apoptotic cells decreased remarkably after interfering with HIPK2 ($p<0.05$). Then, we detected the expression of proliferation and apoptosis (Figure 3D), and found that Ki67 and Bcl-2 protein expression was reduced in the H/R group ($p<0.05$). In contrast, Caspase-3, Caspase-12, and Bax protein expressions were increased ($p<0.05$). In the HIPK2-siRNA + H/R group, the expression of Ki67 and Bcl-2 was increased ($p<0.05$), but the Caspase-3, Caspase-12, and Bax protein expressions decreased ($p<0.05$). Meanwhile, the PCR results were similar to the former (Figure 3E, $p<0.05$).

### HIPK2-siRNA Transfection can Decrease the Phosphorylation Level of JAK2/STAT3 Signaling Pathway Protein Induced by H/R

In our results we detected that the expression of p-JAK2 and p-STAT3 was significantly increased after H/R treatment (Figure 4A and 4B, $p<0.05$), while the expression of p-JAK2 and p-STAT3 in the HIPK2-siRNA + H/R group was remarkably reduced ($p<0.05$). Then, we used WP1066 to inhibit the JAK/STAT pathway and to test the expression of p-JAK2 and p-STAT3 (Figure 4C and 4D). NRK-52E cells were divided as: Control + DMSO, Control + WP1066, H/R + WP1066, HIPK2-siRNA + H/R + WP1066 four groups. We found that compared with the Control + DMSO group, the expression levels of p-JAK2 and p-STAT3 were reduced in the Control + WP1066 group and the H/R + WP1066 group, and the reduction in the Control + WP1066 group

**Table II.** Effect of HIPK-siRNA transfection on NRK-52E cells on H / R-induced 8-OHdG and 5-OH-dC content.

| Experimental group | 8-OHdG/(ng/L) | 5-OH-dC/(pg/mL) |
|---|---|---|
| Control | 12.32 ± 0.23 | 4.23 ± 0.81 |
| H/R | 33.23 ± 3.21* | 14.65 ± 1.88* |
| HIPK2-siRNA+H/R | 22.64 ± 1.88# | 7.21 ± 1.21# |

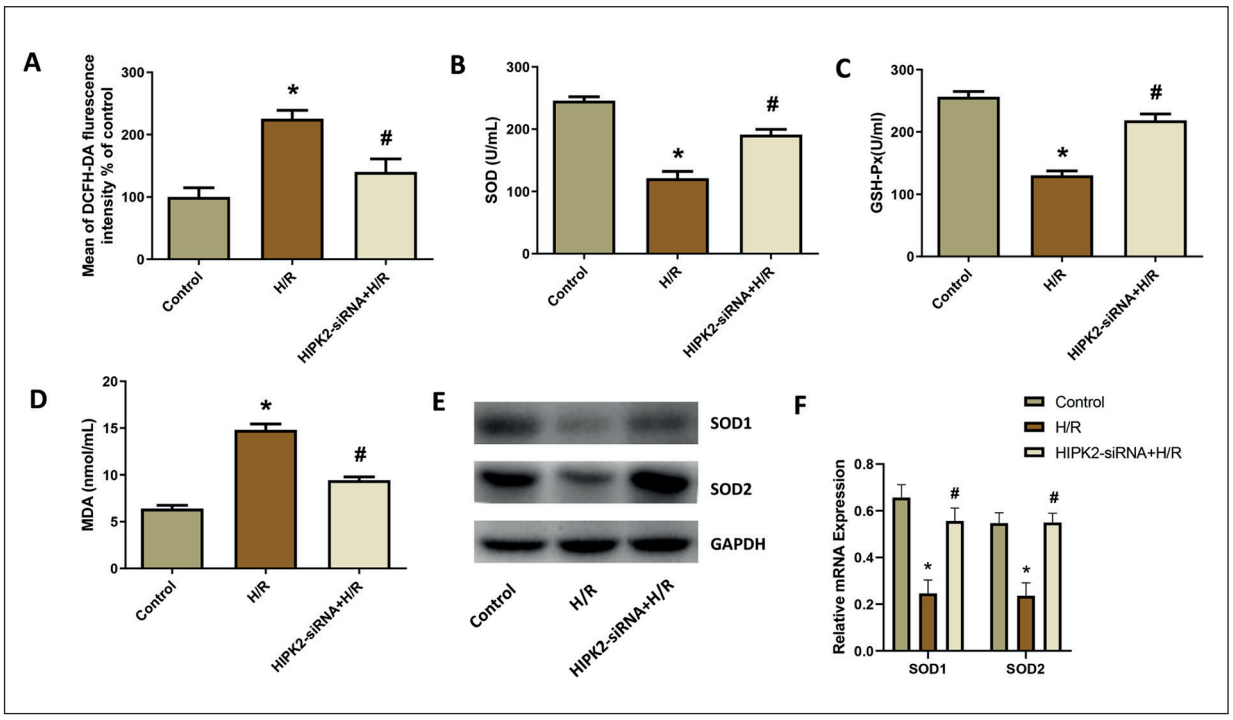


**Figure 2.** Effect of HIPK2-siRNA transfection on H/R induced OS. Detection of 8-OH-dG and 5-OH-dC contents in cell supernatants. **A,** Flow cytometry detection of ROS content. **B,** SOD activity in cell supernatant. **C,** GSH-Px activity in cell supernatant. **D,** MDA content in cell supernatant. **E,** WB detection of SOD1 and SOD2 expression. **F,** PCR detection of SOD1 mRNA and SOD2 mRNA. Mean ± SD, n = 3, "*" $p<0.05$ *vs.* Control group. "#" $p<0.05$ *vs.* H/R group.

was more significant ($p<0.05$). While when we interfered HIPK2, we found that the expression of p-JAK2 and p-STAT3 was decreased more in the HIPK2-siRNA + H/R + WP1066 group, compared with H/R + WP1066 group ($p<0.05$). Therefore, we speculated that the inhibition of HIPK2 expression could decrease the expression of JAK2/STAT3 signaling pathway.

## Discussion

The pathogenesis of renal IRI is complicated, and RTEC apoptosis is an important mechanism, which is closely related to calcium overload, oxygen free radical increase, energy metabolism disorder, and other factors[10]. Therefore, it is particularly important to study the effective treatment methods for renal injury. Homeodomain Interacting Protein Kinases (HIPKs) family is divided into HIPK1, HIPK2, and HIPK3. Mouse HIPK2 gene is located on chromosome 68, and human HIPK2 gene is located at 7 q32-34, which is a protein kinase of silk threonine in the nucleus. HIPK2 is a potential tumor suppressor with multiple functions, which can inhibit tumor growth and enhance drug sensitivity by regulating some key molecular pathways in tumor cells. With the in-depth study of HIPK2, its role in the kidney has been widely concerned. HIPK2 can play a pro-inflammatory and fibrotic role in the kidney by regulating Notch, p53, and Smad3 pathways[11,12]; however, the inhibition of HIPK2 expression can slow down the process of renal fibrosis, which can be used as a therapeutic target for renal fibrosis[13]. Nevertheless, there are few reports on HIPK2 in renal injury.

In this study, HIPK2-siRNA was transfected into NRK-52E cells, and the expression of HIPK2 in the cells was significantly decreased, indicating that the expression of HIPK2 could be dramatically inhibited after transfection. After H/R treatment, the cells were tested for cell viability, OS, and apoptosis by CCK-8 and flow cytometry. The results showed that the cell viability was reduced, the OS was aggravated, and the apoptosis rate was increased. However, after transfection of NRK-52E cells with HIPK2-siRNA, it can significantly improve cell viability, inhibit OS response, and reduce MDA content in lipid oxidation end prod-

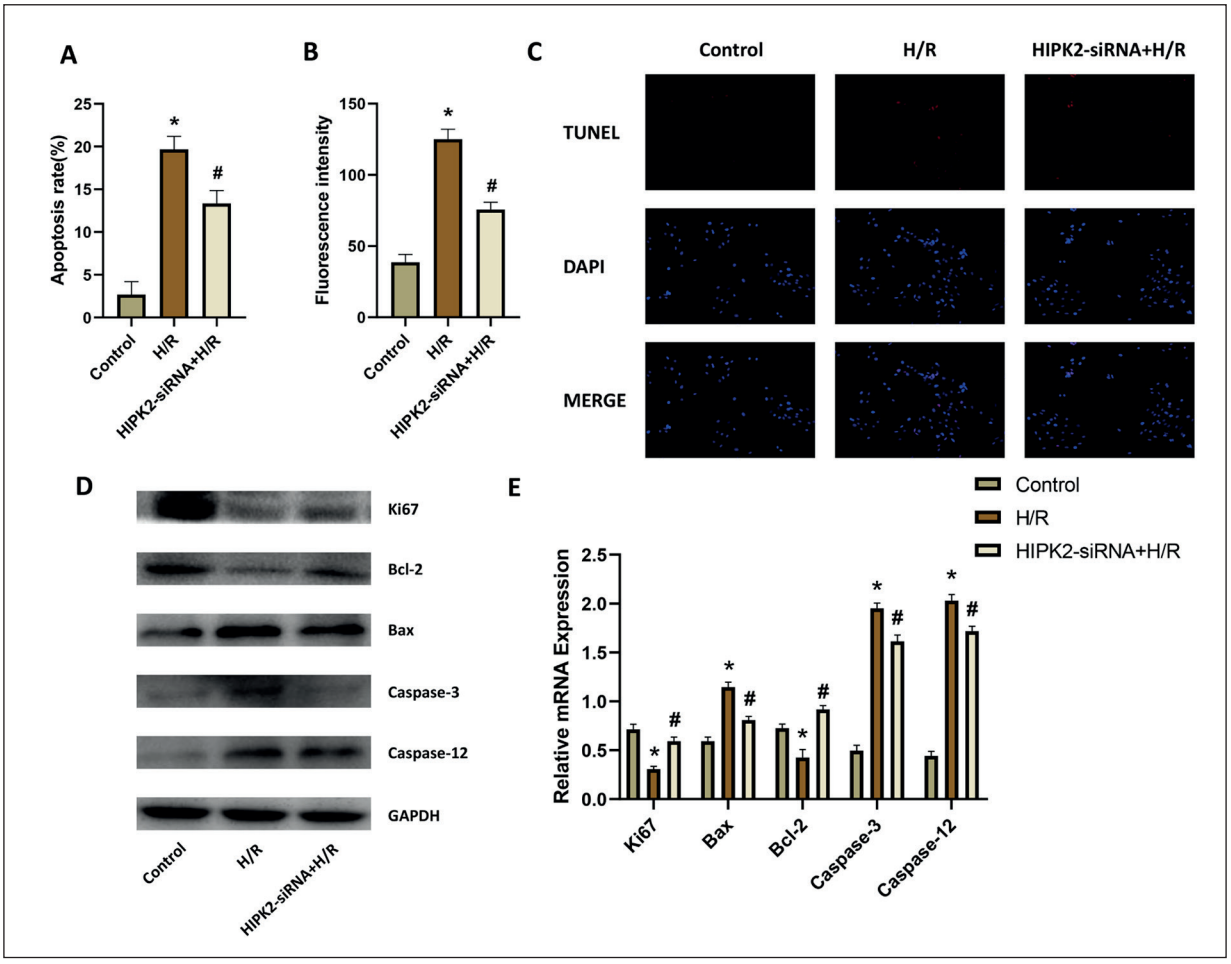


***Figure 3.*** Effect of HIPK2-siRNA transfection on H/R induced proliferation and apoptosis. **A,** Flow cytometry was used to detect the apoptotic rate. **B,** Flow cytometry detection of $Ca^{2+}$ fluorescence. **C,** TUNEL staining (magnification: 400×). **D,** WB detection of Ki67, Bcl-2, Bax, Caspase-3, Caspase-12 expression. **E,** PCR detection of Ki67, Bcl-2, Bax, Caspase-3, Caspase-12 mRNA. Mean ± SD, n = 3, "*" $p<0.05$ *vs.* Control group. "#" $p<0.05$ *vs.* H/R group.

ucts. MDA is also considered as one of the most important signs to judge the level of OS. This suggests that silencing HIPK2 protects the tubules in AKI. As a nuclear antigen related to proliferating cells, Ki67 is an accurate, ideal, and reliable antigen for the detection of cell proliferation activity. It is a cell proliferation-related gene, and the DIVA-binding protein it encodes is an essential substance for cell proliferation. It appears in the middle and late stages of G1, reaches its peak in the M stage after S and G2 stages, and rapidly degrades after the M stage. Therefore, by detecting the expression level of ki67, we can understand the proliferation level of NRK-52E cells[14]. There are two classical pathways of mitochondrial and death receptor. In recent years, it has been detected that excessive endoplasmic reticulum stress (ERS) can cause the initiation of apoptosis, which is a new signaling pathway leading to apoptosis. It can specifically activate Caspase-12, while Caspase-12 can lyse some downstream effector proteases, such as Caspase-3, and eventually cause apoptosis[15,16]. $Ca^{2+}$ can be involved in the regulation of a variety of cellular functions and is an important material basis for the maintenance of normal physiological functions in cells. Wang et al[17] have shown that I/R damage can increase intracellular $Ca^{2+}$ and activate Caspase-12, thereby inducing apoptosis. Our results showed that interfere HIPK2 can promote the proliferation of NRK-52E cells treated with H/R by upregulating Ki67, and inhibited apoptosis by upregulating Bcl-2 expression, downregulating the expression of Caspase-12, Caspase-3 and Bax.

JAK2/STAT3 signaling pathway can regulate a variety of physiological functions, and normal

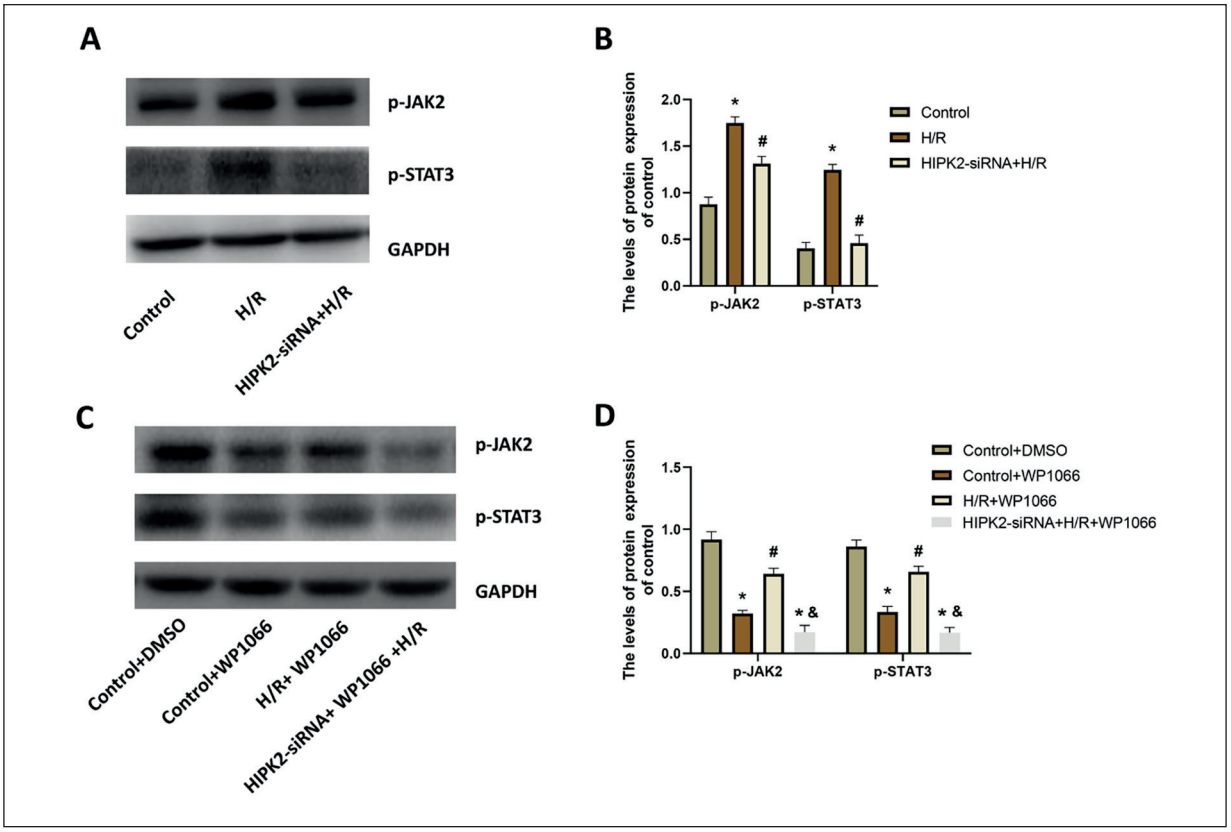


**Figure 4.** HIPK2-siRNA transfection can decrease the phosphorylation level of JAK2 / STAT3 signaling pathway protein induced by H/R. **A,** WB detection of p-JAK2 and p-STAT3 expression. **B,** Protein gray value analysis. Mean ± SD, n = 3, "*" $p<0.05$ *vs.* Control group. "#" $p<0.05$ *vs.* H/R group. **C,** WB detection of p-JAK2 and p-STAT3 expression. **D,** Protein gray value analysis. Mean ± SD, n = 3, "*" $p<0.05$ *vs.* Control + DMSO group, "#" $p<0.05$ *vs.* Control + WP1066 group, "&" $p<0.05$ *vs.* H/R + WP1066 group.

RTEC is in a relatively low level of activation, which mainly mediates repair and proliferation functions[18]. In recent years, the regulatory role in kidney disease has received widespread attention, and it has been proven to be closely related to podocytes, RTEC, and mesangial cells[19]. However, inhibition of JAK2/STAT3 signal can reduce RTEC differentiation[20], H/R environment can activate JAK2/STAT3 signal, and inhibition of its activity will protect RTEC[21]. The results of this research show that inhibition of HIPK2 expression can downregulate JAK2/STAT3 signal expression in NRK-52E cells, which is consistent with previous studies on the role in the kidney.

In the mechanism of renal IRI, different cells of the kidney can show different response patterns; abnormal proliferation of mesangial cells, abnormal synthesis of extracellular matrix (ECM), and abnormally increased secretion are the most important causes of glomerulosclerosis. In the process of renal IRI, the specific mechanism of changes in glomerular epithelial cell (GEC) proliferation and apoptosis has not been fully understood. Therefore, this study aims to explore the impact of renal IRI on RTECs and the role of HIPK2. Of course, we will also carry out experiments on other cells in the future, so as to explore AKD disease more completely.

## Conclusions

The inhibition of HIPK2 gene can promote the growth of NRK-52E RTEC induced by H/R, downregulate the JAK2/STAT3 signaling pathway, alleviate the redox imbalance, reduce the apoptotic rate, and upregulate Ki67, thereby upregulating Bc1-2 expression, and downregulating Caspase-12, Caspase-3, and Bax expression. HIPK2 gene may be a new target for the treatment of AKI. However, *in vivo* studies have

not been conducted in this study, and there are certain limitations, which is also the focus of our future experimental studies.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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## References

1) Levey AS, James MT. Acute kidney injury. Ann Intern Med 2017; 167: C66-C80.
2) Chawla LS, Eggers PW, Star RA, Kimmel PL. Acute kidney injury and chronic kidney disease as interconnected syndromes. N Engl J Med 2014; 371: 58-66.
3) Kang HM, Ahn SH, Choi P, Ko YA, Han SH, Chinga F, Park AS, Tao J, Sharma K, Pullman J, Bottinger EP, Goldberg IJ, Susztak K. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. Nat Med 2015; 21: 37-46.
4) Hashimoto K, Tsuji Y. arsenic-induced activation of the homeodomain-interacting protein kinase 2 (HIPK2) to cAMP-response element binding protein (CREB) axis. J Mol Biol 2017; 429: 64-78.
5) Fan Y, Wang N, Chuang P, He JC. Role of HIPK2 in kidney fibrosis. Kidney Int Suppl (2011) 2014; 4: 97-101.
6) Gao Q, Liang X, Shaikh AS, Zang J, Xu W, Zhang Y. JAK/STAT signal transduction: promising attractive targets for immune, inflammatory and hematopoietic diseases. Curr Drug Targets 2018; 19: 487-500.
7) Darnell JJ, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994; 264: 1415-1421.
8) Smith CC, Dixon RA, Wynne AM, Theodorou L, Ong SG, Subrayan S, Davidson SM, Hausenloy DJ, Yellon DM. Leptin-induced cardioprotection involves JAK/STAT signaling that may be linked to the mitochondrial permeability transition pore. Am J Physiol Heart Circ Physiol 2010; 299: H1265-H1270.
9) Zhang L, Wang Y, Ma J, Lai X, Fang J, Li G, Xu L, Pan G, Chen Z. Exogenous MSCs ameliorate hypoxia/reoxygenation injury in renal tubular epithelial cells through JAK/STAT signaling pathway-mediated regulation of HMGB1. Am J Transl Res 2017; 9: 2412-2420.
10) Yang Y, Zhang ZX, Lian D, Haig A, Bhattacharjee RN, Jevnikar AM. IL-37 inhibits IL-18-induced tubular epithelial cell expression of pro-inflammatory cytokines and renal ischemia-reperfusion injury. Kidney Int 2015; 87: 396-408.
11) Huang Y, Tong J, He F, Yu X, Fan L, Hu J, Tan J, Chen Z. MiR-141 regulates TGF-beta1-induced epithelial-mesenchymal transition through repression of HIPK2 expression in renal tubular epithelial cells. Int J Mol Med 2015; 35: 311-318.
12) Xiong C, Masucci MV, Zhou X, Liu N, Zang X, Tolbert E, Zhao TC, Zhuang S. Pharmacological targeting of BET proteins inhibits renal fibroblast activation and alleviates renal fibrosis. Oncotarget 2016; 7: 69291-69308.
13) Chuang PY, Menon MC, He JC. Molecular targets for treatment of kidney fibrosis. J Mol Med (Berl) 2013; 91: 549-559.
14) Ellis MJ, Suman VJ, Hoog J, Goncalves R, Sanati S, Creighton CJ, DeSchryver K, Crouch E, Brink A, Watson M, Luo J, Tao Y, Barnes M, Dowsett M, Budd GT, Winer E, Silverman P, Esserman L, Carey L, Ma CX, Unzeitig G, Pluard T, Whitworth P, Babiera G, Guenther JM, Dayao Z, Ota D, Leitch M, Olson JJ, Allred DC, Hunt K. Ki67 proliferation index as a tool for chemotherapy decisions during and after neoadjuvant aromatase inhibitor treatment of breast cancer: results from the American College of Surgeons Oncology Group Z1031 Trial (Alliance). J Clin Oncol 2017; 35: 1061-1069.
15) Huang Y, Li X, Wang Y, Wang H, Huang C, Li J. Endoplasmic reticulum stress-induced hepatic stellate cell apoptosis through calcium-mediated JNK/P38 MAPK and Calpain/Caspase-12 pathways. Mol Cell Biochem 2014; 394: 1-12.
16) Goswami P, Gupta S, Biswas J, Joshi N, Swarnkar S, Nath C, Singh S. Endoplasmic reticulum stress plays a key role in rotenone-induced apoptotic death of neurons. Mol Neurobiol 2016; 53: 285-298.
17) Wang Y, Wei S, Wang YL, Liu M, Shang M, Zhang Q, Wu YN, Liu ML, Song JQ, Liu YX. Protective effects of circulating microvesicles derived from myocardial ischemic rats on apoptosis of cardiomyocytes in myocardial ischemia/reperfusion injury. Oncotarget 2017; 8: 54572-54582.
18) Miao L, Liu K, Xie M, Xing Y, Xi T. MiR-375 inhibits Helicobacter pylori-induced gastric carcinogenesis by blocking JAK2-STAT3 signaling. Cancer Immunol Immunother 2014; 63: 699-711.
19) Yu J, Wu H, Liu ZY, Zhu Q, Shan C, Zhang KQ. Advanced glycation end products induce the apoptosis of and inflammation in mouse podocytes through CXCL9-mediated JAK2/STAT3 pathway activation. Int J Mol Med 2017; 40: 1185-1193.
20) Wang HY, Zhang C, Xiao QF, Dou HC, Chen Y, Gu CM, Cui MJ. Hepatocyte growth factor inhibits tubular epithelial myofibroblast transdifferentiation by suppression of angiotensin II via the JAK2/STAT3 signaling pathway. Mol Med Rep 2017; 15: 2737-2743.
21) Lv J, Wang X, Liu SY, Liang PF, Feng M, Zhang LL, Xu AP. Protective effect of fenofibrate in renal ischemia reperfusion injury: involved in suppressing kinase 2 (JAK2)/transcription 3 (STAT3)/p53 signaling activation. Pathol Biol (Paris) 2015; 63: 236-242.