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Journal

The FASEB Journal, 36(11)

ISSN

0892-6638

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Publication Date

2022-11-01

DOI

10.1096/fj.202101626rr

Received: 20 October 2021 | Revised: 24 August 2022 | Accepted: 29 September 2022

DOI: 10.1096/fj.202101626RR

THE FASEB JOURNAL

RESEARCH ARTICLE

Dexmedetomidine attenuates renal ischemia–reperfusion injury through activating PI3K/Akt-eNOS signaling via α_2 adrenoreceptors in renal microvascular endothelial cells

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Funding information
National Natural Science Foundation of China (NSFC), Grant/Award Number: 82072130 and 81873925; Natural Science Foundation of Jiangsu Province (Jiangsu Natural Science Foundation), Grant/Award Number:

Abstract

Renal microvascular endothelial cells (RMECs), which are closely related to regulation of vascular reactivity and modulation of inflammation, play a crucial role in the process of renal ischemia and reperfusion (I/R) injury. Previous studies have reported the protective effects of dexmedetomidine (DEX) against renal I/R injury, but little is known about the role of DEX on RMECs. This study aimed to investigate whether DEX alleviated renal I/R injury via acting on the RMECs. Mice underwent bilateral renal artery clamping for 45 min followed by reperfusion for 48 h, and the cultured neonatal mice RMECs were subjected to hypoxia for 1 h followed by reoxygenation (H/R) for 24 h. The results suggest that DEX alleviated renal I/R injury in vivo and improved cell viability of RMECs during H/R injury in vitro. Gene sequencing revealed that the PI3K/Akt was the top enriched signaling pathway and the endothelial cells were widely involved in renal I/R injury. DEX activated phosphorylation of PI3K and Akt, increased eNOS expression, and attenuated inflammatory responses. In addition, the results confirmed the distribution of α_2 adrenoreceptor (α_2-AR) in RMECs. Furthermore, the protective effects of DEX against renal I/R injury were abolished by α_2-AR antagonist (atipamezole), which was partly reversed by the PI3K agonist (740 Y-P). These findings indicated that DEX protects against renal I/R injury by activating the PI3K/Akt-eNOS pathway and inhibiting inflammation responses via α_2-AR in RMECs.

Abbreviations: AKI, acute kidney injury; Atip, atipamezole; BUN, blood urea nitrogen; CCK-8, cell counting kit-8; ceRNA, competitive endogenous RNA; circRNA, circular RNA; DEGs, differentially expressed genes; DEX, dexmedetomidine; ECGS, endothelial cell growth supplement; eNOS, endothelial NOS; FBS, fetal bovine serum; FDR, false discovery rate; GO, Gene Ontology; H&E, hematoxylin and eosin; H/R, hypoxia and reoxygenation; I/R, ischemia/reperfusion; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; iNOS, inducible NOS; KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long noncoding RNA; MCP-1, monocyte chemoattractant protein-1; miRNA, microRNA; mRNA, messenger RNA; nNOS, neuronal NOS; NO, nitric oxide; NOS, NO synthase; p-Akt, phospho-Akt; p-PI3K, phospho-PI3K; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol 3-hydroxykinase; PVDF, polyvinylidene fluoride; RMECs, renal microvascular endothelial cells; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; vWF, von willebrand factor; α_2-AR, α_2 adrenoreceptor.

Xisheng Shan, Jiaxin Zhang, and Xiang Wei contributed equally to this study.

BK20191171; Six Talent Peaks Project in Jiangsu Province (Jiangsu Province Six-Category Talent Program), Grant/Award Number: WSN-022; Clinical Trial Project of Science and Technology Development Plan in Suzhou city, Grant/Award Number: SLT201909; 333 High-level Talent Training Project in Jiangsu Province, Grant/Award Number: BRA2020089; Natural Science Foundation of the Jiangsu Higher Education Institutions of China, Grant/Award Number: 22KJD320002

KEYWORDS

dexmedetomidine, endothelial nitric oxide synthase, microvascular endothelial cell, PI3K/Akt, renal ischemia and reperfusion injury, α_2 adrenoreceptors

1 | INTRODUCTION

Renal ischemia and reperfusion (I/R) injury, as an unavoidable clinical complication during kidney transplantation and cardiopulmonary resuscitation in perioperative period, is a major cause of acute kidney injury (AKI).[1] Renal I/R injury-induced AKI results in a rapid decline in kidney function, finally leading to chronic kidney disease and end-stage kidney disease.[2] Currently, several interventions have been used in clinical therapy. However, AKI is still associated with prolonged hospitalization, enhanced medical expenditure, and high mortality rates.[3,4] Therefore, it has important significance for potential AKI patients to alleviate renal I/R injury.

Increasing evidence indicates that I/R injury is associated with endothelial damage, especially in the peritubular capillary network.[5,6] Some studies suggest that renal I/R injury causes disruptions of the perivascular matrix, leading to increased endothelial barrier permeability.[7,8] Besides acting as a barrier, renal microvascular endothelial cells (RMECs) also adjust blood flow distribution, modulate inflammatory responses, and regulate vascular permeability.[9] The RMECs dysfunctions induced I/R injury contributes to the decreased renal perfusion, continued renal hypoxia, and subsequent tubular epithelial cell injury.[6,10] Thus, RMECs injury plays a crucial role in the pathogenesis of I/R injury-induced AKI. However, the mechanisms of RMEC dysfunction during renal I/R injury are not fully understood.

Dexmedetomidine (DEX) is a highly selective α_2-adrenoreceptor(α_2-AR) agonist with sedative, analgesic, sympatholytic, anti-inflammatory, and hemodynamic stabilizing effects.[11] Some previous evidence indicates that inflammatory responses play crucial roles in the pathogenesis of I/R injury, and inflammatory cascades are induced by the endothelial cell injury.[8,12] As well known, DEX could improve perioperative renal function by regulating the α2-AR.[13,14] Indeed, α2-AR is widely expressed in the peritubular vasculature and vascular endothelium. One study has confirmed that DEX protects brain tissue against I/R injury through activating phosphatidylinositol 3-hydroxykinase (PI3K)/Akt signaling pathway.[15] In addition, DEX regulated vasodilation by increasing the endothelial nitric oxide synthase (eNOS) expression in endothelial cell.[16] Thus, the aim of the current study is to investigate DEX reduces renal I/R injury through activating PI3K/Akt-eNOS signaling in RMECs via regulating α_2-AR.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult healthy male C57BL/6J mice weighing 20–25 g were obtained from the Experimental Animal Centre of Soochow University (Animal license No. SYXK Jiang-su 2017-0043). Animals received standard diets under a controlled environment with 12-h light/dark cycle, 40%–60% relative humidity, and room temperature of 24–26°C. The animal experimental protocol was approved by the Ethics Committee for Animal Experimentation of Soochow University. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996).

2.2 | Renal I/R injury model

The renal I/R injury was induced as previously described.[17] The mice were intraperitoneally anesthetized with 40 mg/kg sodium pentobarbital. The bilateral renal pedicles occlusion for ischemia for 45 min was performed using microvascular clamps, and then the artery clamps were removed for reperfusion for 48 h. Sham group mice underwent the same surgical procedures but with no occlusion of the renal pedicles. The intrarectal temperature of all animals was maintained at 37°C by using a heating pad and a rectal thermometer. The animals were sacrificed and kidney tissues were harvested at 48 h after the surgery.

2.3 | Isolation and culture of renal microvascular endothelial cells

The renal microvascular endothelial cells (RMECs) were obtained according to the previously described.[18,19] Briefly, kidney tissues were dissected out from neonatal mice on postnatal days 1–2 and transferred to ice-cold phosphate-buffered saline (PBS) solution under aseptic conditions. The tissues were mechanically minced and placed into digestion solution containing collagenase type IV (1.0 mg/ml, Sigma, St. Louis, MO, USA) and DNase (0.5 mg/ml, Sigma-Aldrich; St. Louis, MO, USA) for 40 min at 37°C. Afterward, the cellular suspension was filtered through cell strainer (BD Biosciences, San Diego, CA, USA) in order to obtain a single cellular mixture, centrifuged at 300 *g* for 10 min and resuspended in the Endothelial Cell Medium containing 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA), 5 ng/ml vascular endothelial growth factor (VEGF, Sigma-Aldrich; St. Louis, MO, USA), 1% endothelial cell growth supplement (ECGS; Sigma-Aldrich; St. Louis, MO, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. After 48–72 h in culture, the cells are removed using trypsin to produce a suspension of single cells. Then, the RMECs were isolated using CD31 MicroBeads (MACS, Miltenyi Biotechnology, Bergisch Gladbach, Germany) according to the manufacturer's protocol. First, the cell pellet was added 90 μl of autoMACS® Rinsing Solution buffer and 10 μl of CD31 MicroBeads per 10^7 total cells. After incubating for 15 min at 4°C, the cells were washed by adding 2 ml of buffer, centrifuged at 300 *g* for 10 min and resuspend with 500 μl of buffer. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD31+ cells are retained within the column and the unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained CD31+ cells can be eluted by firmly pushing the plunger into the column. Finally, the isolated RMECs were resuspended in the cultured media and cultured in a humidified incubator (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C with 5% CO_2. After the third generation, the cultured cells were used for subsequent experiments.

2.4 | Hypoxia/reoxygenation (H/R) model

To establish the hypoxia/reoxygenation (H/R) model in vitro, the RMECs were incubated treated with glucose-free DMEM and incubated at 37°C for 1 h in a hypoxia chamber containing 95% N_2 and 5% CO_2. For reoxygenation, the cells were transferred to the fresh normal DMEM and cultured for 48 h.

2.5 | Experimental protocols

The assessments of this study were made by an investigator who was blinded to the protocol including in vivo and in vitro experiments. And the protocol was shown in Figure 1.

In vivo Part I: To examine the protective effects of DEX against renal I/R injury, the mice were randomly divided into three groups: Sham group, I/R group, and DEX + I/R group. Besides, the mice in DEX + I/R group were received intraperitoneal injection of DEX (50 μg/kg, Jiangsu Hengrui Medicine Co, Ltd.) at two different time points. The first injection was 30 min prior to ischemia, and the second injection was at the end of the ischemia.

Part II: To investigate the protective role of DEX against renal I/R injury through activating PI3K/Akt-eNOS signaling via α2-AR, atipamezole (Atip, 250 μg/kg, abs816081, Absin) as an α2-AR antagonist and 740 Y-P (2.5 mg/kg, B5426, APExBIO, Houston, TX, USA) as the PI3K agonist were chosen to use during I/R injury with DEX administration. The mice were randomly divided into five groups: Sham group, I/R group, DEX + I/R group, Atip + DEX + I/R group, and Atip + 740Y-P + DEX + I/R group.

In vitro Part I: To determine the optimal concentration of DEX against H/R injury, the cultured RMECs were randomly divided into five groups: the control group, the H/R group, and DEX + H/R groups (cells were treated with 0.1, 1, and 10 μM DEX, respectively).

Part II: To examine the protective effects of DEX against RMECs H/R injury, the cells were randomly divided into three groups: control group, H/R group, and DEX + H/R group. Besides, the cultured RMECs in DEX + I/R group were received DEX treatment at two different time points. The first time point was 24 h prior to hypoxia, and the second time point was at the beginning of the reoxygenation.

Part III: To investigate the protective role of DEX against H/R injury through activating PI3K/Akt-eNOS signaling via α2-AR, 10 μM Atip and 20 μM 740 Y-P were chosen to use during H/R injury with DEX treatment. The cells were randomly divided into five groups: control group, H/R group, DEX + H/R group, Atip+DEX + H/R group, and Atip + 740Y-P + DEX + H/R group.

2.6 | Histopathological evaluation of kidney

For histopathological assessment, kidney tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Then, 4 μm thick sections were prepared and

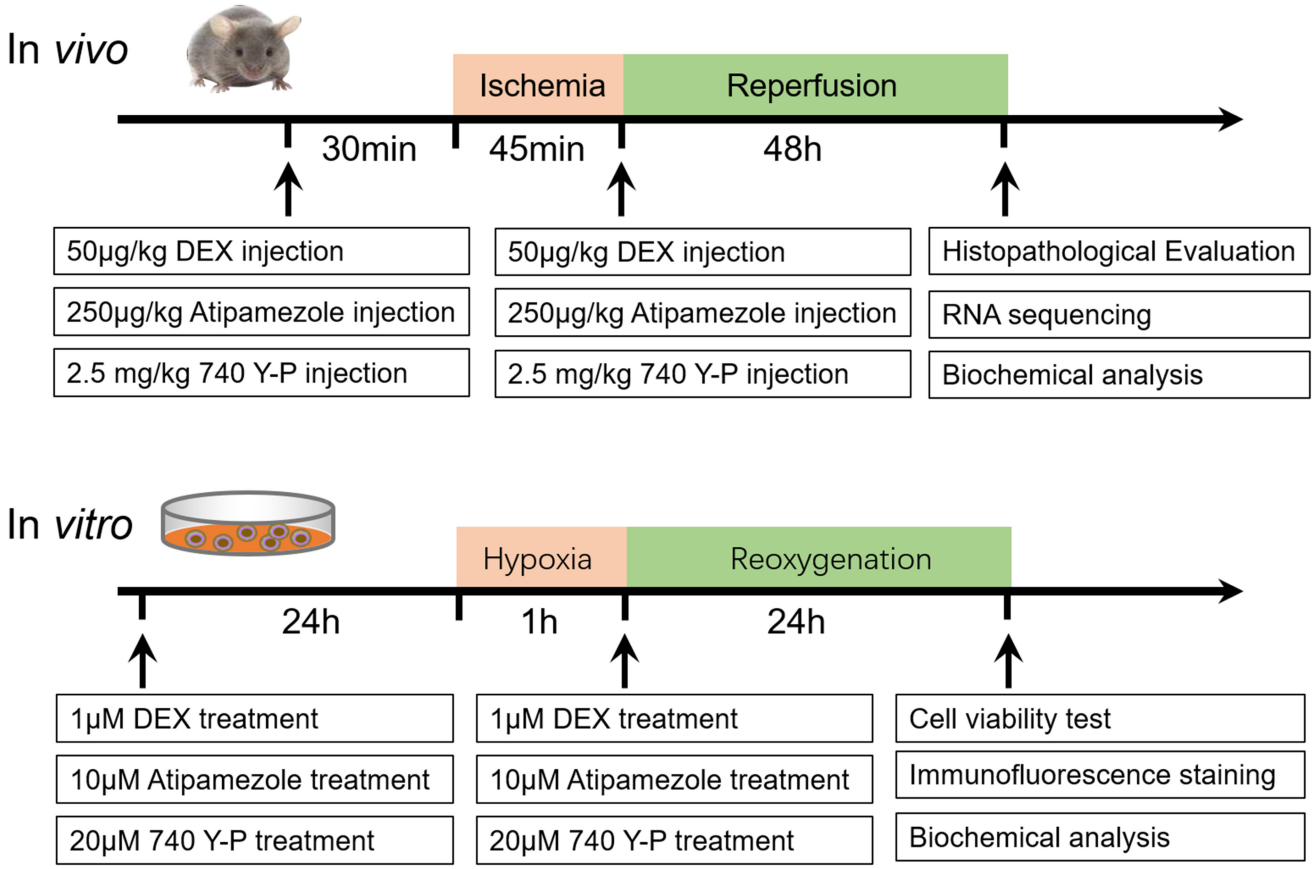

FIGURE 1 Experimental protocols. Mice underwent bilateral renal artery clamping for 45 min followed by reperfusion for 48 h, and neonatal mice RMECs were subjected to hypoxia for 1 h followed by reoxygenation (H/R) for 24 h. Gene sequencing of kidney tissues was implemented to uncover relevant signaling pathways. Renal function, histological parameters, cell viability, immunofluorescence staining, p-PI3K, p-Akt, and eNOS protein levels, NO concentration, and related inflammatory factors mRNA levels were assessed.

stained with hematoxylin and eosin (H&E). The histopathological evaluation was performed under light microscopy, histological lesion was graded on a scale of 0 to 4 as previously described[17]: 0 = normal kidney; 1 = minimal damage (<5% involvement of the cortex or outer medulla); 2 = mild damage (5%–25% involvement of the cortex or outer medulla); 3 = moderate damage (25%–75% involvement of the cortex or outer medulla); 4 = severe damage (>75% involvement of the cortex or outer medulla).

2.7 | Masson trichrome staining

Masson trichrome staining was performed to evaluate collagen fibrils in renal tissues. Samples from paraffin-embedded tissues were prepared at 4 μm thickness and stained with Masson's trichrome stain kit (Absin Bioscience, Inc., Shanghai, China) following the standard protocols. For quantification, at least ten different fields were randomly selected from each section and analyzed by Image-Pro Plus software. A ratio of blue stained area to the area of entire field was assessed and expressed as percentage of fibrotic area.

2.8 | Immunohistochemical staining

The paraffin-embedded sections with 4 μm thickness were used for immunohistochemical staining. First, the sections were deparaffinized in xylene and rehydrated in graded alcohol. Then, endogenous peroxidase was incubated with 3% H_2O_2 for 30 min at room temperature, and the sections were blocked with 10% goat serum in Tris-buffered saline for 30 min at 37°C. Subsequently, the sections were incubated with primary antibodies against α-SMA (1:200 dilution; Abcam, USA) at 4°C for overnight, followed by incubation with an HRP-conjugated secondary antibody at room temperature for 30 min. After incubation with the chromogenic substrate using a peroxidase substrate-diaminobenzidine kit (Beyotime Institute of Biotechnology, Shanghai, China), sections were counterstained in hematoxylin followed by gradient dehydration and were resin sealed. For the quantification, at least ten different fields were randomly selected

from each section and counted the positive staining signal using ImageJ software (National Institutes of Health, Bethesda, MD, United States).

2.9 | Serum creatinine and blood urea nitrogen assay

After reperfusion for 48 h, animals were sacrificed and the blood samples were centrifuged at 1000 *g* for 10 min at 4°C. The serum was collected for biochemical analysis. Serum creatinine (ab65340, Abcam, Cambridge, MA, USA) and blood urea nitrogen (BUN, ab83362, Abcam, Cambridge, MA, USA) reagent kits were performed according to the manufacturer's instructions, respectively. The absorbance values were measured by using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.10 | RNA extraction and sequencing

Total RNA was extracted from kidney tissues using Trizol reagent kit (Invitrogen; Thermo Fisher Scientific, MA, USA) according to the manufacturer's protocol. RNA quality was assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and checked using RNase-free agarose gel electrophoresis. Sequencing library was constructed by using the TruSeq RNA preparation kit (Illumina, San Diego, CA, USA), following the manufacturer's protocols.

2.11 | Differentially expressed mRNA, miRNA, lncRNA, and circRNA

To identify differentially expressed transcripts across samples or groups, the edgeR package (http://www.bioconductor.org/packages/release/bioc/html/edgeR.html) was used. We identified the differentially expressed mRNA and lncRNA with $|\text{Log}_2$ fold change$| \geq 1$ and false discovery rate (FDR)-adjusted p value <.05 in a comparison between groups. Besides, the criteria of $|\text{Log}_2$ fold change$| \geq 1$ and p value <.05 was used to screened the differentially expressed miRNA and circRNA.

2.12 | Functional annotation of DEGs and ceRNA construction

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed to analyze the enriched signaling pathways of DEGs based on the KEGG database (http://www.genome.jp/kegg/). Gene Ontology (GO) analysis was performed to assess the functional enrichment of DEGs in the biological process based on the Gene Ontology database (http://www.geneontology.org/).

Expression correlation between lncRNA-miRNA, circRNA-miRNA, and miRNA-mRNA was evaluated using Spearman's Rank correlation coefficient. The lncRNA/circRNA-miRNA-mRNA network was constructed by assembling all co-expression competing triplets and was visualized using Cytoscape software (v3.6.0) (http://www.cytoscape.org/).

2.13 | Quantitative real-time PCR

Total RNA from kidney tissues were extracted by using Trizol reagent and the cDNA was synthetized with using the reverse transcription kits (Applied Biological Materials, Richmond, BC, Canada). Quantitative PCR was conducted with EvaGreen qPCR MasterMix (Applied Biological Materials, Richmond, BC, Canada) in 10 μl reaction volumes on Roche Light Cycler R480 System (Roche, Bedford, MA, USA). And 10 μl reaction volume contained 1.5 μl of cDNA, 5 μl Eva Green, 1 μl of each pair of primers, and 1.5 μl DEPC water. The reaction was performed for 40 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and elongation at 72°C for 32 s. Three replicates were applied for each sample. The target gene expression was normalized to GAPDH and analyzed by $2^{-\Delta\Delta CT}$ method. The sequences of the primers were listed in Table 1.

2.14 | Detection of nitric oxide (NO)

According to the manufacturer's instructions, the NO levels in kidney tissues and RMECs were determined by detection of the stable NO metabolites nitrite and nitrate with using Nitric Oxide Assay Kit (ab65328, Abcam, Cambridge, MA, USA). The final absorbance values were measured at 540 nm wavelength using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.15 | Western blot

The total protein in kidney tissues and renal microvascular endothelial cells were extracted by using the ice-cold lysis buffer supplemented with RIPA reagents (P0013B, Beyotime, Shanghai, China). And the protein concentration was determined with Bicinchoninic Acid Protein Assay Kit (P1002, Beyotime, Shanghai, China). Afterward, the proteins were separated by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10%–12% gels and transferred to polyvinylidene fluoride (PVDF)

TABLE 1 The sequences of primers

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
PI3K	AAACAAAGCGGAGAACCTATTG	TAATGACGCAATGCTTGACTTC
Akt	TGCACAAACGAGGGGAATATAT	CGTTCCTTGTAGCCAATAAAGG
eNOS	CTGTGGCTGTGGAACAACTGGAG	CGAAGGAGGCGAGGACTAGGC
p53	TGGAAGGAAATTTGTATCCCGA	GTGGATGGTGGTATACTCAGAG
NF-κB	CAAAGACAAAGAGGAAGTGCAA	GATGGAATGTAATCCCACCGTA
FOXO4	CTTCCTCGACCAGACCTCG	ACAGGATCGGTTCGGAGTGT
Bax	TCCTCACTGCCTCACTCACCATC	CCTTTCCCCGTTCCCCATTCATC
Bcl-2	TGGAGAGCGTCAACAGGGAGATG	GGTGTGCAGATGCCGGTTCAG
TNF-α	ATGTCTCAGCCTCTTCTCATTC	GCTTGTCACTCGAATTTTGAGA
IL-6	ACTTCCAGCCAGTTGCCTTCTTG	TTAAGCCTCCGACTTGTGAAGTGG
ICAM-1	CTGAAAGATGAGCTCGAGAGTG	AAACGAATACACGGTGATGGTA
MCP-1	TTTTTGTCACCAAGCTCAAGAG	TTCTGATCTCATTTGGTTCCGA
GAPDH	GGTTGTCTCCTGCGACTTCA	AGCCCAGGATGCCCTTTAGT

membrane (ISEQ00010, Millipore Corp., Bedford, MA). The membrane was blocked for 2 h, incubated with the primary antibodies overnight at 4°C, and then incubated with the goat anti-mouse or goat anti-rabbit secondary antibodies for 2 h at room temperature. Bands were visualized by a ChemiDocTM XRS+ System (Bio-Rad, Richmond, CA) with an enhanced chemiluminescence kit (New Cell&Molecular, Shanghai, China). Finally, the densities of protein were normalized to GAPDH as control. The primary antibodies were used as the following: mouse anti-eNOS (1:1000, ab76198, Abcam), rabbit anti-α_2-AR (1:1000, abs133387, Absin, Shanghai, China), rabbit anti-p-PI3K (1:1000, 17366S, Cell Signaling Technology, Beverly, MA, USA), rabbit anti-PI3K (1:1000, 4249S, Cell Signaling Technology), rabbit anti-p-Akt (1:1000, 4060S, Cell Signaling Technology), and rabbit anti-Akt (1:1000, 4691S, Cell Signaling Technology).

2.16 | Cell viability

Cell viability was evaluated by using the Cell Counting Kit-8 (CCK-8) assay (Beyotime, Shanghai, China) as previously described.[20] Briefly, RMECs were cultured in 96-well plates. After reoxygenation, 10 μl CCK8 solution was added to each well and incubated for 1 h at 37°C. The absorbance values at 450 nm were measured by using the microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.17 | Immunofluorescence staining

RMECs were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100 and then blocked with 10% normal donkey serum for 2 h at room temperature. For the purity identification of RMECs, the formaldehyde-fixed cells were incubated with the rabbit anti-Von Willebrand Factor (vWF, 1:500, ab154193, Abcam) in a humidified chamber overnight at 4°C, and then incubated with Alexa Fluor® 594-conjugated AffiniPure Donkey anti-Rabbit IgG (711-585-152, Jackson). Afterward, the cell nuclei were stained with DAPI (Beyotime, Shanghai, China).

Besides, the double immunofluorescence staining of formaldehyde-fixed cells was performed using rabbit anti-vWF (1:500, ab154193, Abcam) and rabbit anti-α2-AR (1:500, abs133387, Absin) in humidified chamber overnight at 4°C, then the cells were incubated by Alexa Fluor® 488-conjugated AffiniPure Fab Fragment Donkey anti-Rabbit IgG (711-547-003, Jackson) and Alexa Fluor® 594-conjugated AffiniPure Donkey anti-Rabbit IgG (711-585-152, Jackson) for 2 h at room temperature, respectively. Images were captured using a fluorescence microscope (Nikon Corporation; Tokyo, Japan) and analyzed with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

2.18 | Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM) and analyzed using the GraphPad Prism software (version 7.0, GraphPad, San Diego, CA, USA). Statistical significance was determined by using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, as appropriate. $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | DEX administration improved histological injury and renal function during renal I/R injury

To confirm the effect of DEX on kidney tissue in mice during renal I/R injury, we performed the H&E, Masson

trichrome, and immunohistochemistry staining. H&E staining showed that normal morphology of tubular architecture was observed in the sham group, while renal I/R injury resulted in severe tubular damage, the destruction included widespread degeneration of tubular architecture, tubular dilation, tubular cell swelling, pyknotic nuclei, and luminal congestion. DEX administration significantly improved tubular damage in cortical and medullar in kidney tissues induced by I/R (Figure 2A). The renal I/R injury significantly increased the histopathological scores compared to the sham group, and DEX administration effectively reduced the increased histopathological score during I/R injury (Figure 2B). Masson trichrome staining was also carried out to observe the tubular interstitial fibrosis (Figure 2A). The results showed that the fibrosis areas in I/R group were more than that in sham groups, and DEX treatment significantly reduced the fibrosis areas in kidney tissues during renal I/R injury (Figure 2C). The expression of α-SMA, as the fibrosis markers, was further detected using immunohistochemistry staining (Figure 2A). DEX significantly inhibited the upregulated positive expression of α-SMA during I/R injury (Figure 2D). In addition, similar changes were also found in renal function. The mice subjected to I/R had dramatic increase in serum creatinine and BUN level compared to the mice in sham group. Besides, DEX treatment significantly decreased serum creatinine and blood urea nitrogen level during renal I/R injury (Figure 2E,F).

3.2 | Identification of DEGs and KEGG pathway analysis in renal I/R injury

Our RNA-seq analysis showed that renal I/R altered the expression level of 22 299 genes in the mice kidney tissues compared to the Sham mice. Of these genes, a total of 2486 DEGs were discovered, including 1901 up-regulated genes and 585 down-regulated genes in the heatmaps (Figure 3A) and volcano plot (Figure 3B) in the kidney tissues of I/R mice. To further analyze the enriched signaling pathways of DEGs in renal I/R injury, KEGG analyses were performed using the KEGG database (Figure 3C). Among the enriched signaling pathways, PI3K/Akt signaling ranked forefront in the injury-related pathways. Several DEGs from RNA-seq at the top of injury-related pathway were verified by qPCR. We found that the I/R injury significantly increased the PI3K, Akt, eNOS, TNF-α, IL-6, and NF-κB mRNA levels (Figure 3D–I). However,

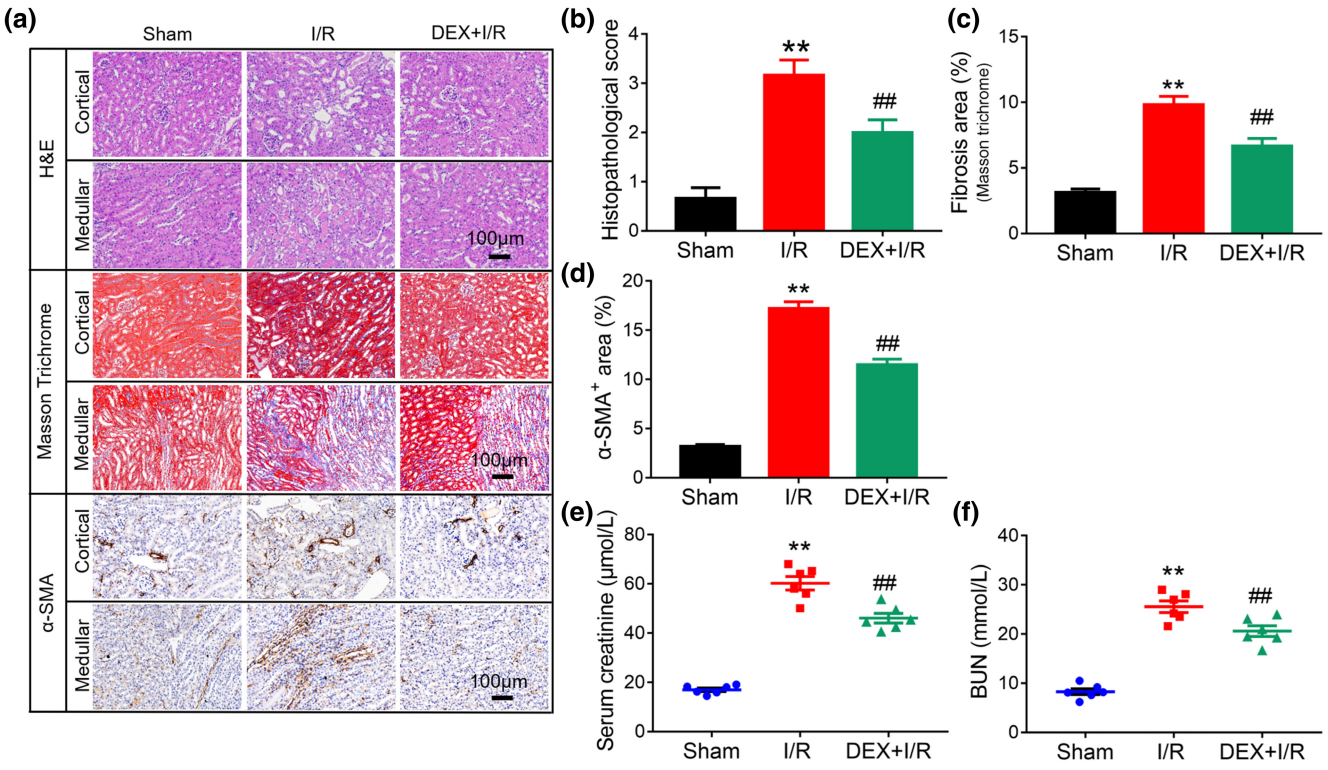

FIGURE 2 DEX administration improved histological injury and renal function during renal I/R injury. (A) DEX improved tubular damage in cortical and medullar in kidney tissues induced by I/R; (B) DEX decreased the elevated histopathological scores induced by I/R; (C) DEX reduced the enhanced fibrosis area induced by I/R; (D) DEX inhibited the upregulated positive expression of α-SMA induced by I/R; (E) DEX reduced the increased serum creatinine level induced by I/R; (F) DEX inhibited the enhanced BUN level induced by I/R. Values were expressed as the mean ± SEM ($n = 6$ for each group). $^{**}p < .01$ vs. Sham; $^{\#\#}p < .01$ vs. I/R.

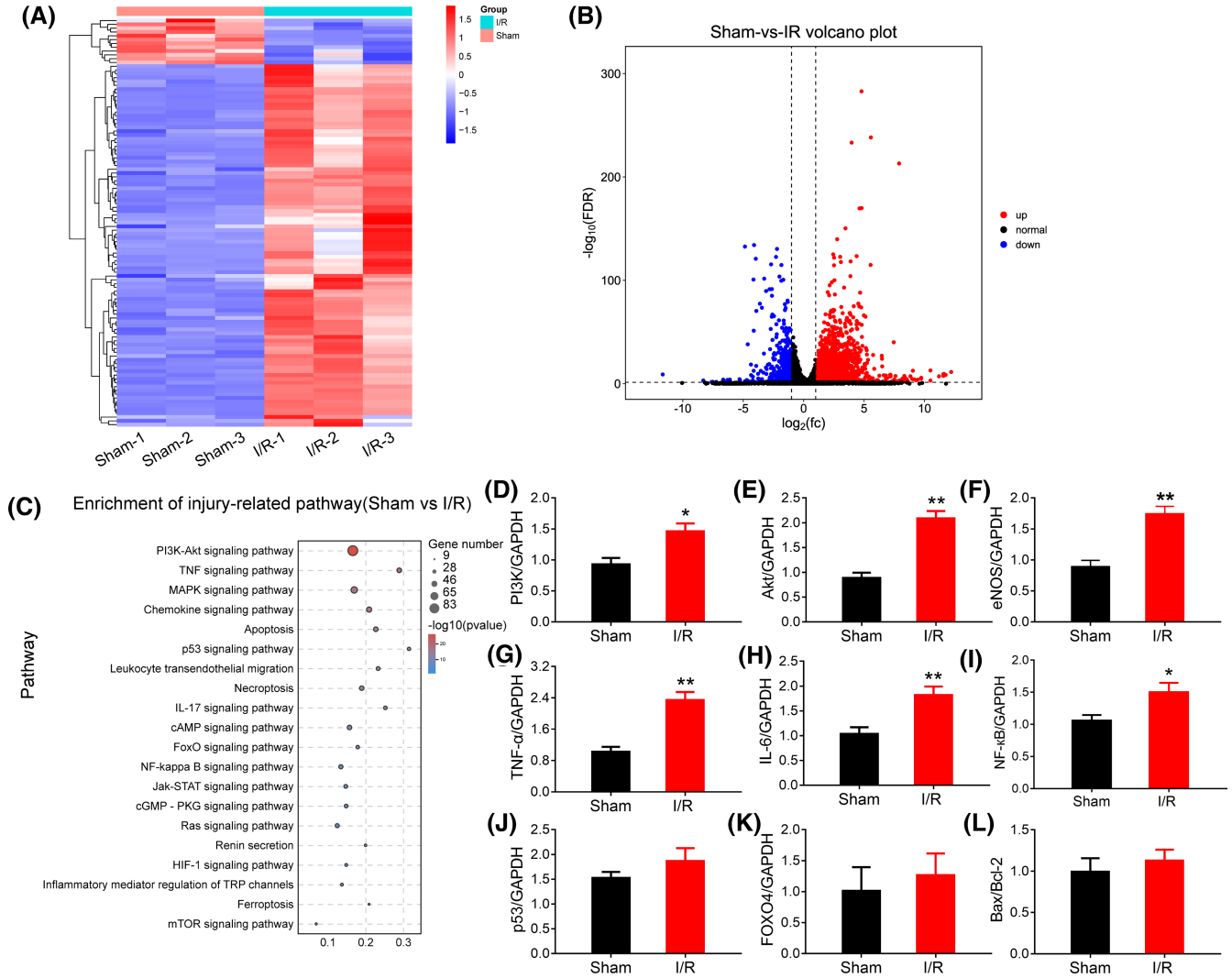

FIGURE 3 Identification of DEGs and KEGG Pathway analysis in renal I/R injury. (A) Heatmap showing 1901 up-regulated DEGs (red) and 585 down-regulated DEGs (green). (B) Volcano plot showing up-regulated (red dots) and down-regulated (blue dots) and normally expressed genes (black dots). (C) KEGG enrichment showing top 20 injury-related pathways. (D–I) I/R increased PI3K, Akt, eNOS, TNF-α, IL-6, and NF-κB mRNA levels ($n = 6$ for each group); (J) p53 mRNA level was not altered during I/R; (K) FOXO4 mRNA level was not altered during I/R; (L) Bax/Bcl-2 was not altered during I/R; ($n = 5$ for each group). Values were expressed as the mean ± SEM. *$p < .05$, **$p < .01$ vs. Sham.

there was no significant difference in the p53, FOXO4, and Bax/Bcl2 mRNA levels between I/R and sham groups (Figure 3J–L).

3.3 | DEX activated PI3K/Akt-eNOS pathway and attenuated inflammatory response during renal I/R injury

Based on the crucial role of PI3K/Akt signaling in the renal I/R injury, we further investigated whether PI3K/Akt and eNOS were involved in DEX-elicited protection against renal I/R injury. Western blotting analysis showed that I/R injury significantly increased the p-PI3K, p-Akt, and eNOS protein expression. Moreover, DEX treatment further enhanced these above protein expressions (Figure 4A–D). Besides, there was no significant change in total PI3K and Akt after I/R injury or DEX treatment. We also found that I/R significantly increased the inflammatory factors, intercellular adhesion molecule, and chemokines in kidney tissue, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1) mRNA levels, while were blocked by DEX administration (Figure 4E–H).

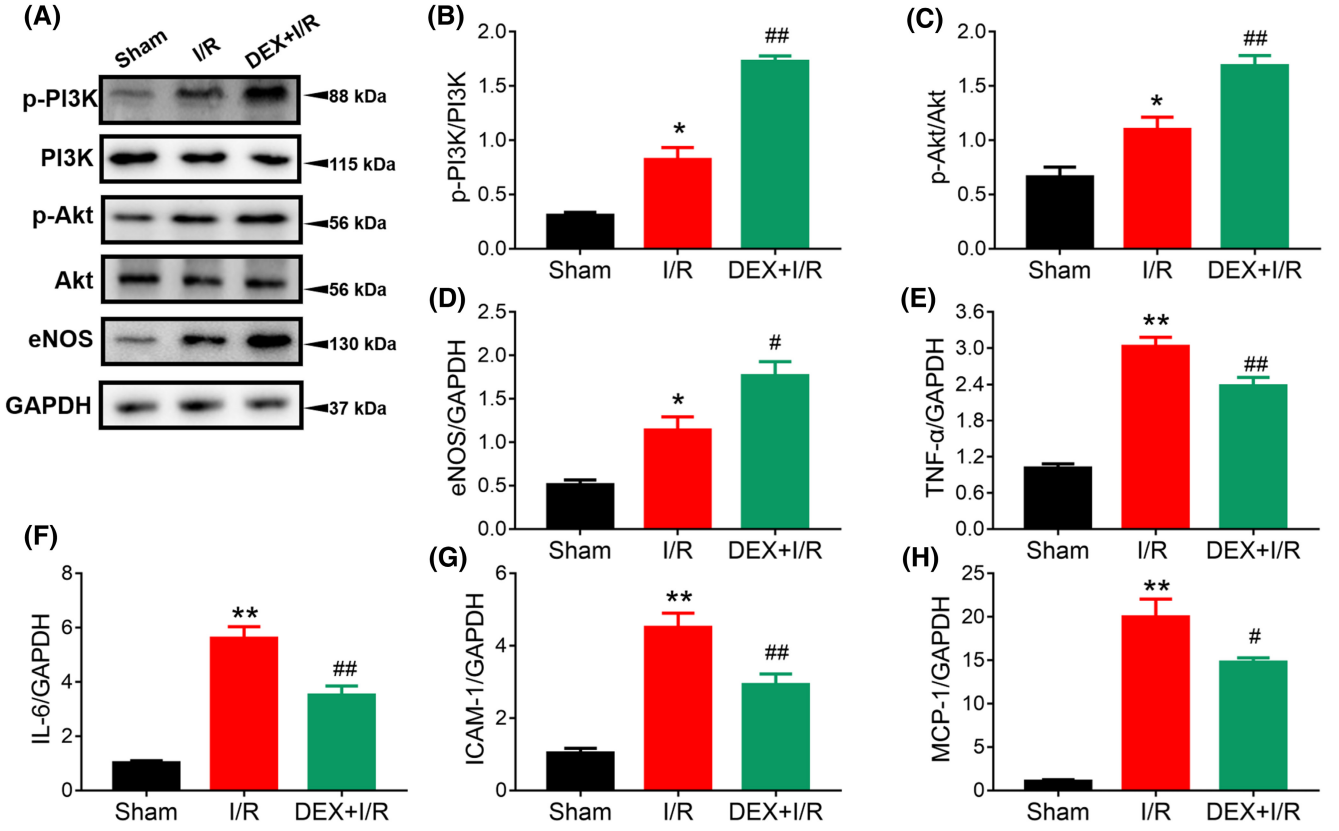

FIGURE 4 DEX activated PI3K/Akt-eNOS pathway and attenuated inflammatory response during renal I/R injury. (A) The protein levels were detected by western blotting; (B–D) DEX further increased p-PI3K, p-Akt, and eNOS protein level induced by I/R injury ($n = 4$ for each group); (E–H) DEX reversed TNF-α, IL-6, ICAM-1, and MCP-1 mRNA levels induced by I/R injury ($n = 6$ for each group). Values were expressed as the mean ± SEM. $^{**}p < .01$ vs. Sham; $^{\#}p < .05$, $^{\#\#}p < .01$ vs. I/R.

3.4 | The protective effect of DEX against renal I/R injury through activating PI3K/Akt-eNOS signaling via α_2-AR

The results showed that an α_2-AR antagonist Atip effectively blocked the elevated protein expressions of p-PI3K, p-Akt, and eNOS, as well as the increased NO production, induced by DEX treatment during I/R injury. We also found that PI3K agonist, 740 Y-P, significantly reversed p-PI3K, p-Akt, and eNOS protein expressions induced by Atip and DEX treatment during renal I/R injury (Figure 5A–D). Besides, 740 Y-P administrations significantly reversed the NO production (Figure 5E), morphology of tubular architecture in cortical and medullar (Figure 5F), as well as the histopathological scores, the fibrosis areas and the positive expression of α-SMA (Figure 5G–I), in the kidney tissues during mice I/R injury treated with Atip and DEX. These results indicated that DEX administration enhanced the PI3K/Akt-eNOS signaling pathway during I/R injury by regulating α2-AR.

3.5 | DEX alleviated H/R induced injury in RMECs

In order to investigate the role of RMECs in the pathogenesis of I/R injury, we examined the RNA sequencing results and found that endothelial cells were widely involved in renal I/R injury, and the DEX administration significantly altered the biological processes of endothelial cells induced by I/R injury. GO enriched analysis was carried out and showed the top 20 endothelial cell interactions of biological processes after renal I/R or DEX treatment (Figure 6A,B). And top 3 enriched GO terms related to the endothelial cell were endothelial cell proliferation, regulation of vascular endothelial growth factor production, and regulation of endothelial cell proliferation. Then, we further demonstrated the role of DEX on the cultured RMECs during H/R injury. The neonatal mouse kidney tissues was chosen to isolate RMECs in our current study considering that some previous reports demonstrated the isolation of endothelial cells from neonatal mice kidney tissues that can be reliably cultured to at least passage eight, enable a more complete digestion of the tissue

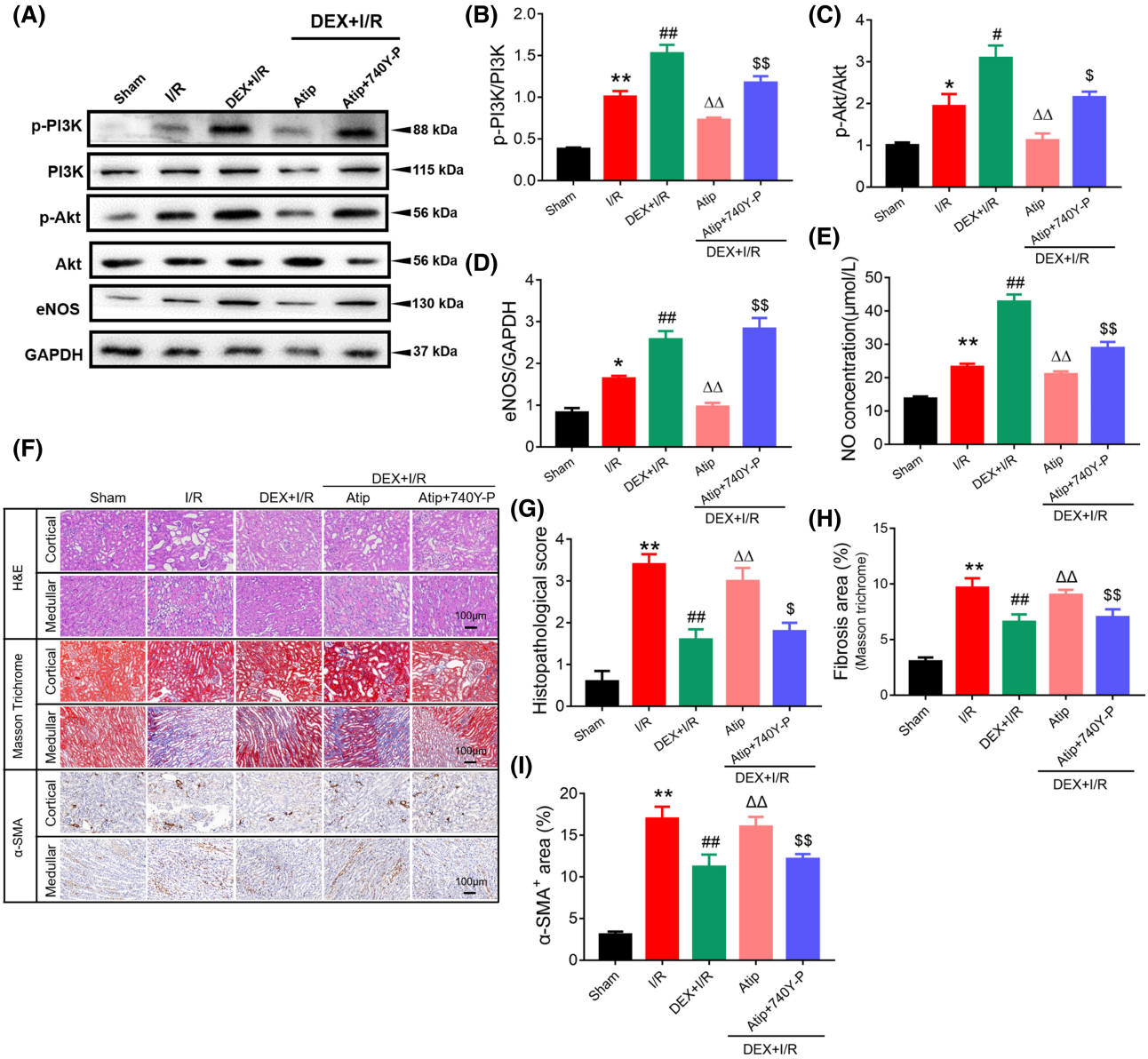

FIGURE 5 The protective effect of DEX against renal I/R injury through activating PI3K/Akt-eNOS signaling via α_2-AR. (A) The protein levels were detected by western blotting; (B–D) p-PI3K, p-Akt, and eNOS protein expression ($n = 4$ for each group); (E) NO concentration in the kidney tissue ($n = 6$ for each group); (F) H&E, Masson trichrome, and immunohistochemistry staining; (G) Histopathological scores ($n = 6$ for each group). (H) Fibrosis areas ($n = 6$ for each group). (I)The positive expression of α-SMA ($n = 6$ for each group). Values were expressed as the mean ± SEM. $^{*}p < .05$, $^{**}p < .01$ vs. Sham; $^{\#}p < .05$, $^{\#\#}p < .01$ vs. I/R; $^{\Delta\Delta}p < .01$ vs. DEX + I/R; $^{\$}p < .05$, $^{\$\$}p < .01$ vs. Atip + DEX + I/R.

and dispersal of the cells, produce more viable cells after sorting, and minimize fibroblast contamination, but the application of the procedures to isolate endothelial cells from older or adult mice results in cultures that are more susceptible to fibroblast overgrowth and the isolation of ECs that rapidly become senescent.[21,22] The vWF mainly produced by endothelial cells was regarded as a promising biomarker for endothelial dysfunction.[23] Thus, the purity of RMECs harvested from the kidney tissue in neonatal mice was identified by the immunofluorescence staining with vWF (Figure 6C). And our results showed that the purity of cultured RMECs was 92.42% (Figure 6D). H/R significantly reduced RMECs viability and the lowest cell viability was induced by the H1/R24 treatment (Figure 6E). Thus, this time-point was selected for the subsequent cell experiments. Then, mice RMECs were respectively incubated with 0.1, 1, or 10 μM DEX, and the results indicated that DEX treatment significantly increased the cell viability during H/R injury (Figure 6F). The use of 0.1 μM DEX was less effective than the 1 μM DEX, and there was no significant difference between 1 and 10 μM DEX. Besides, 1 μM DEX had no impact on cell viability in

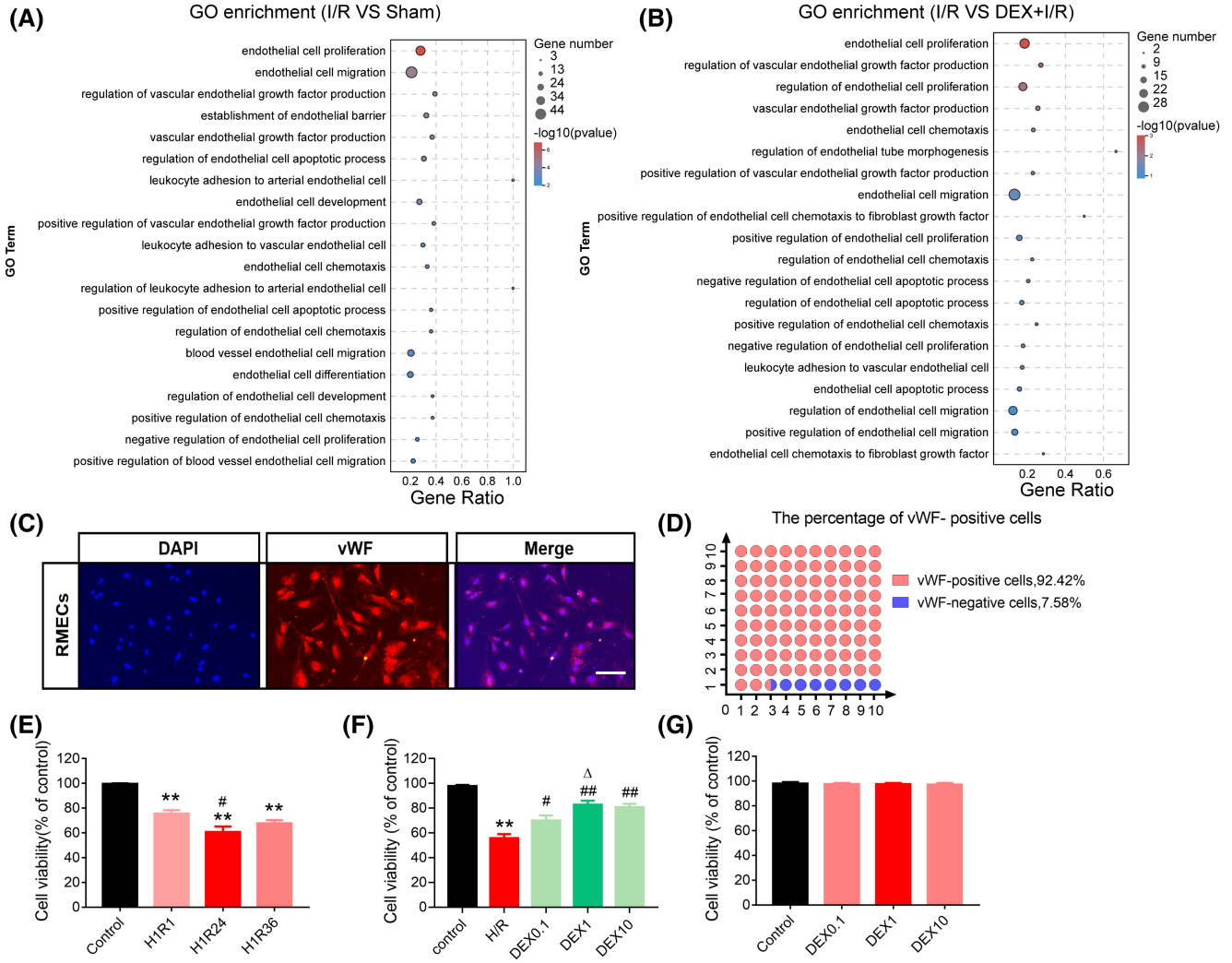

FIGURE 6 DEX alleviated H/R-induced injury in RMECs. (A) GO enrichment of DEGs related endothelial cell from I/R vs. Sham. (B) GO enrichment of DEGs related endothelial cell from DEX + I/R vs. I/R. (C) Immunofluorescence staining with vWF in RMECs, scale bar = 100 μm. (D) The percentage of positive RMECs. (E) H/R significant decrease cell viability ($n = 6$ for each group). (F) DEX treatment enhanced cell viability during RMECs H/R injury ($n = 9$ for each group). (G) DEX had no effect on cell viability in normal cultured RMECs ($n = 6$ for each group). Values were expressed as the mean ± SEM. $^{*}p < .05$, $^{**}p < .01$ vs. Control; $^{\#}p < .05$, $^{\#\#}p < .01$ vs. H1R1 or H/R; $^{\Delta}p < .05$ vs. DEX 0.1.

normal cultured RMECs (Figure 6G). Based on these results, 1 μM DEX was used in the subsequent experiments.

3.6 | DEX activated PI3K/Akt-eNOS pathway and attenuated inflammatory response during RMECs H/R injury

H/R treatment significantly increased the p-PI3K, p-Akt, and eNOS protein expression in RMECs compared to the control group. And DEX administration further enhanced the p-PI3K, p-Akt, and eNOS protein levels during H/R injury (Figure 7A–D). Besides, there was no significant change in total PI3K and Akt after H/R injury or DEX treatment. We also found that H/R significantly increased the inflammatory factors, intercellular adhesion molecule, and chemokines in kidney tissue, including TNF-α, IL-6, ICAM-1, and MCP-1mRNA levels, while was partly reversed by DEX administration (Figure 7E–H).

3.7 | The protective effect of DEX against RMEC H/R injury through activating PI3K/Akt-eNOS signaling via α_2-AR

To investigate whether DEX-elicited protection against H/R in RMECs through regulating the α_2-AR, double labeling studies were performed in the RMECs. As shown in Figure 8A, α_2-AR-positive cells (green) and

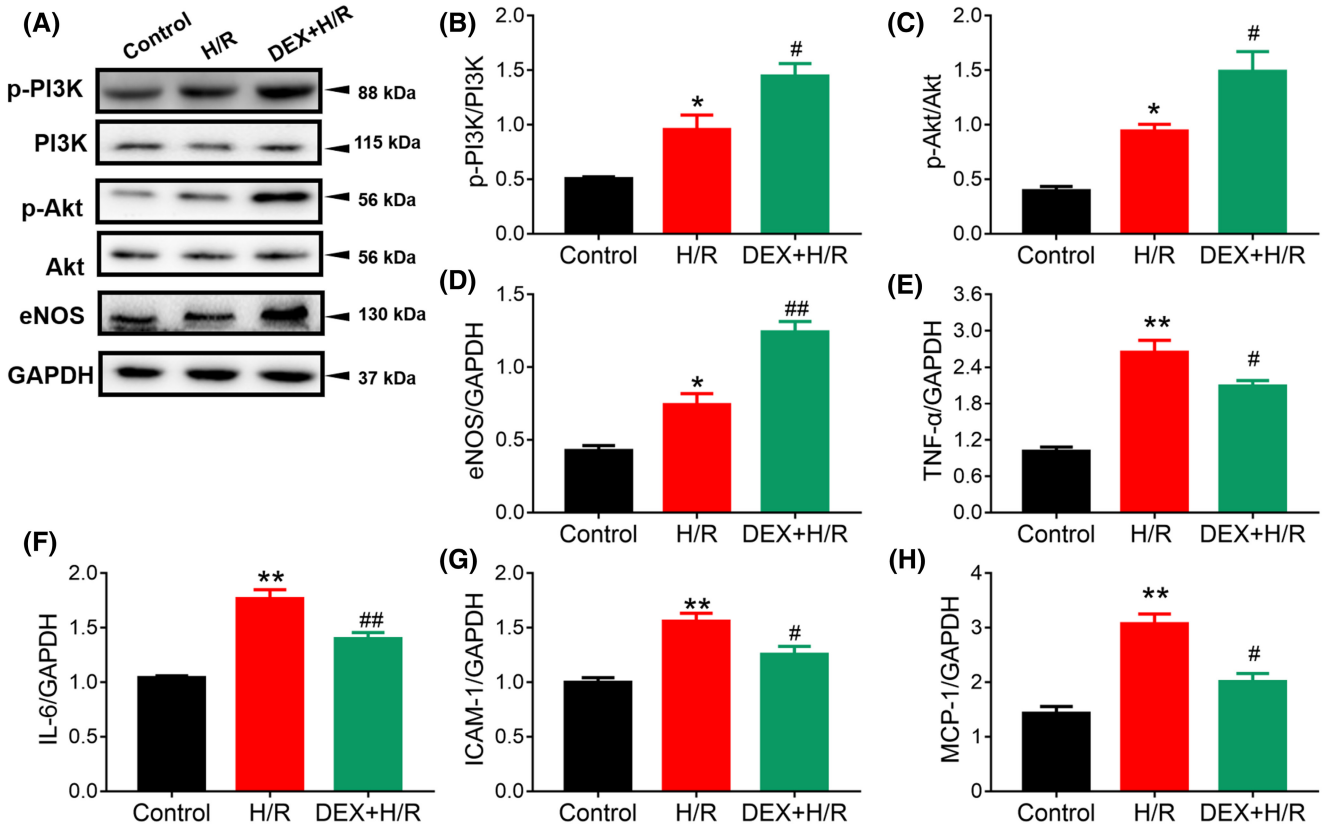

FIGURE 7 DEX activated PI3K/Akt-*eNOS* pathway and attenuated inflammatory response during RMECs H/R injury. (A) The related protein levels were detected by western blotting; (B–D) DEX further increased p-PI3K, p-Akt, and eNOS protein expression during I/R ($n = 4$ for each group); (E–F) DEX decreased TNF-α and IL-6 mRNA level ($n = 6$ for each group); (G-H) DEX treatment reduced ICAM-1 and MCP-1 mRNA level ($n = 6$ for each group). Values were expressed as the mean ± SEM. $^{*}p < .05$, $^{**}p < .01$ vs. Control; $^{\#}p < .05$, $^{\#\#}p < .01$ vs. H/R.

vWF-positive cells (red) colocalized in the RMECs (yellow), and the percentage of α_2-AR-positive cells in RMECs were 96.44% (Figure 8B). Atip effectively blocked the elevated protein expressions of p-PI3K, p-Akt, and e-NOS protein expression, reduced the elevated NO production and inhibited the decreased inflammatory response in RMECs induced by DEX treatment during H/R injury. In addition, 740 Y-P as a PI3K agonist significantly reversed the above protein expressions induced by Atip and DEX treatment during RMECs H/R injury (Figure 8C–F). 740 Y-P also significantly reversed the NO production, cell viability, TNF-α, IL-6, ICAM-1, and MCP-1mRNA level during mice I/R injury treated with Atip and DEX (Figure 8G–L).

3.8 | The ceRNA network associated with PI3K/Akt signaling pathway was regulated by DEX during renal I/R injury

Our present study confirmed that DEX exerted renal protection by activating the PI3K/Akt-eNOS signaling pathway in microvascular endothelial cells. However, the upstream regulatory mechanism of DEX targeting PI3K/Akt-eNOS signaling was still unclear. The RNA-seq analysis showed that total of 44 DEGs related to PI3K/Akt signaling pathway were regulated by DEX during renal I/R injury, including six up-regulated genes and 38 down-regulated genes (Figure 9A), and the gene list was shown in Table 2. Then, we constructed the ceRNA network by integrating the lncRNA/circRNA-miRNA-mRNA interactions (Figure 9B,C). lncRNAs, circRNAs, and miRNAs in the network were listed in the Tables S1, respectively.

4 | DISCUSSION

DEX has been widely used for long-term sedation and analgesia in aesthesia. In addition to its sedative effect, the previous research observed in animal experiments indicated that DEX could protect kidney against I/R injury.[17,24] Our recent clinical study indicated that DEX reduced the incidence of AKI following endovascular aortic repair procedures in Stanford type B aortic dissection patients.[25] Some studies have demonstrated that PI3K/Akt signaling

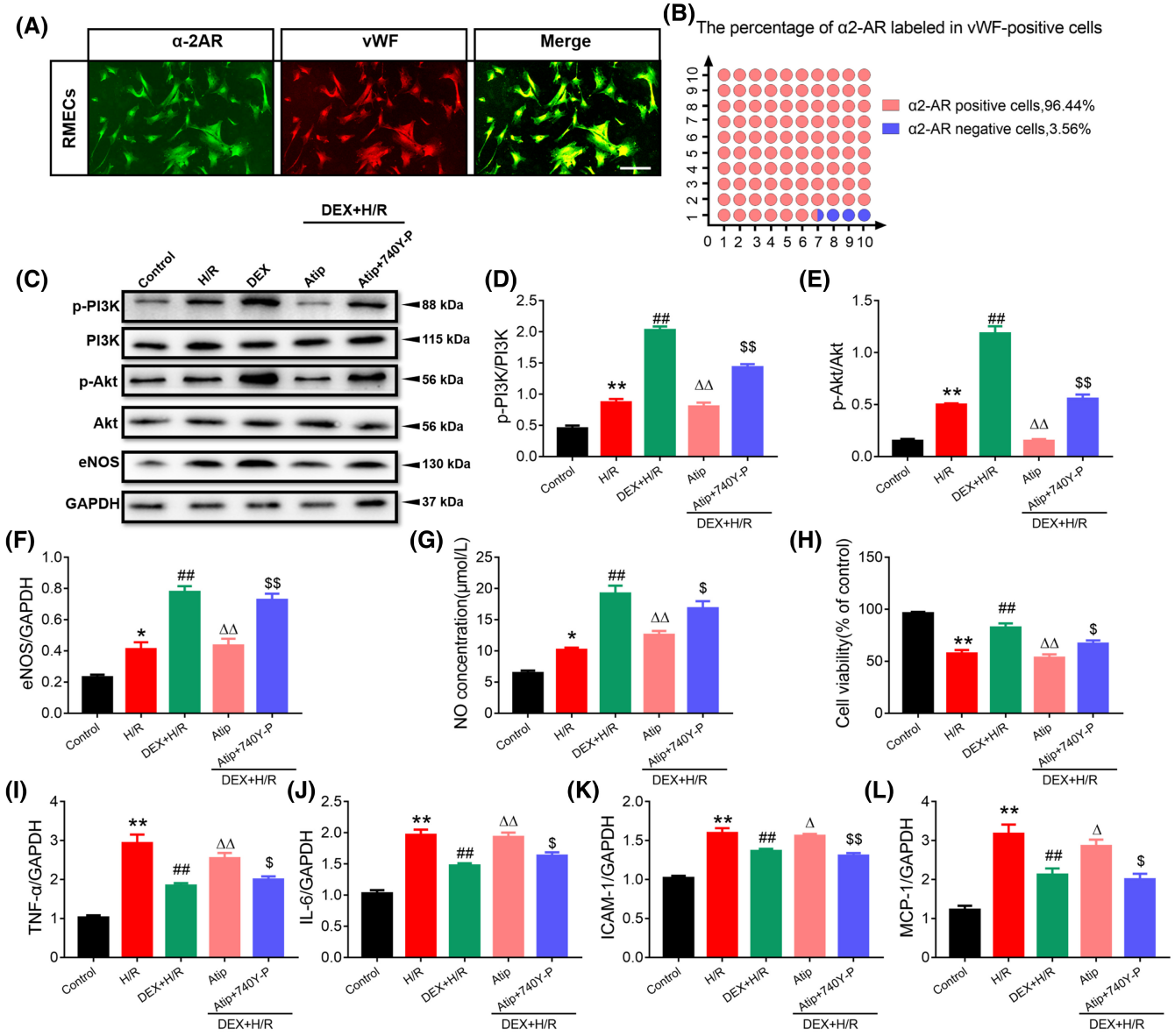

FIGURE 8 The protective effect of DEX against RMECs H/R injury through activating PI3K/Akt-eNOS signaling via α_2-AR. (A) α_2-AR and vWF were colocalized in the RMECs, scale bars = 100 μm; (B) The percentage of α_2-AR labeled in vWF positive cells; (C–F) p-PI3K, p-Akt, and eNOS protein expression ($n = 4$ for each group); (G) NO concentration in cultured RMECs ($n = 6$ for each group); (H) Cell viability in cultured RMECs ($n = 7$ for each group); (I,J) TNF-α and IL-6 mRNA in cultured RMECs ($n = 6$ for each group); (K,L) ICAM-1 and MCP-1 mRNA in cultured RMECs ($n = 6$ for each group). Values were expressed as the mean ± SEM. $^{*}p < .05$, $^{**}p < .01$ vs. Control; $^{\#\#}p < .01$ vs. H/R; $^{\Delta}p < .05$, $^{\Delta\Delta}p < .01$ vs. DEX + H/R; $^{\$}p < .05$, $^{\$\$}p < .01$ vs. Atip + DEX + H/R.

pathway was involved in I/R injury through regulating inflammatory response.[26–28] Microvascular endothelial cells participated in the vascular reactivity and regulated the inflammatory processes in acute kidney injury.[5] One recent study has demonstrated that PI3K/Akt signaling plays an important protective role in mediating endothelial regeneration and vascular repair in the response to organ injury.[29,30] Based on these current findings, the aim of this present study is to investigate the exact protective effect of DEX on microvascular endothelial cells during renal I/R injury. To the best of our current knowledge, this is the first study to demonstrate that DEX reduced renal I/R injury by activating PI3K/Akt-eNOS signaling via regulating α_2-AR in renal microvascular endothelial cells (Figure 10).

Perioperative DEX reduced the kidney injury in patients undergoing cardiac surgery and other major surgical procedures.[25,31] And some animal studies found that renal I/R resulted in severe tubular damage,[32,33] which were consistent with our present findings that the mice subjected to I/R had dramatic increase in serum creatinine and blood urea nitrogen level. In addition, we found that DEX treatment

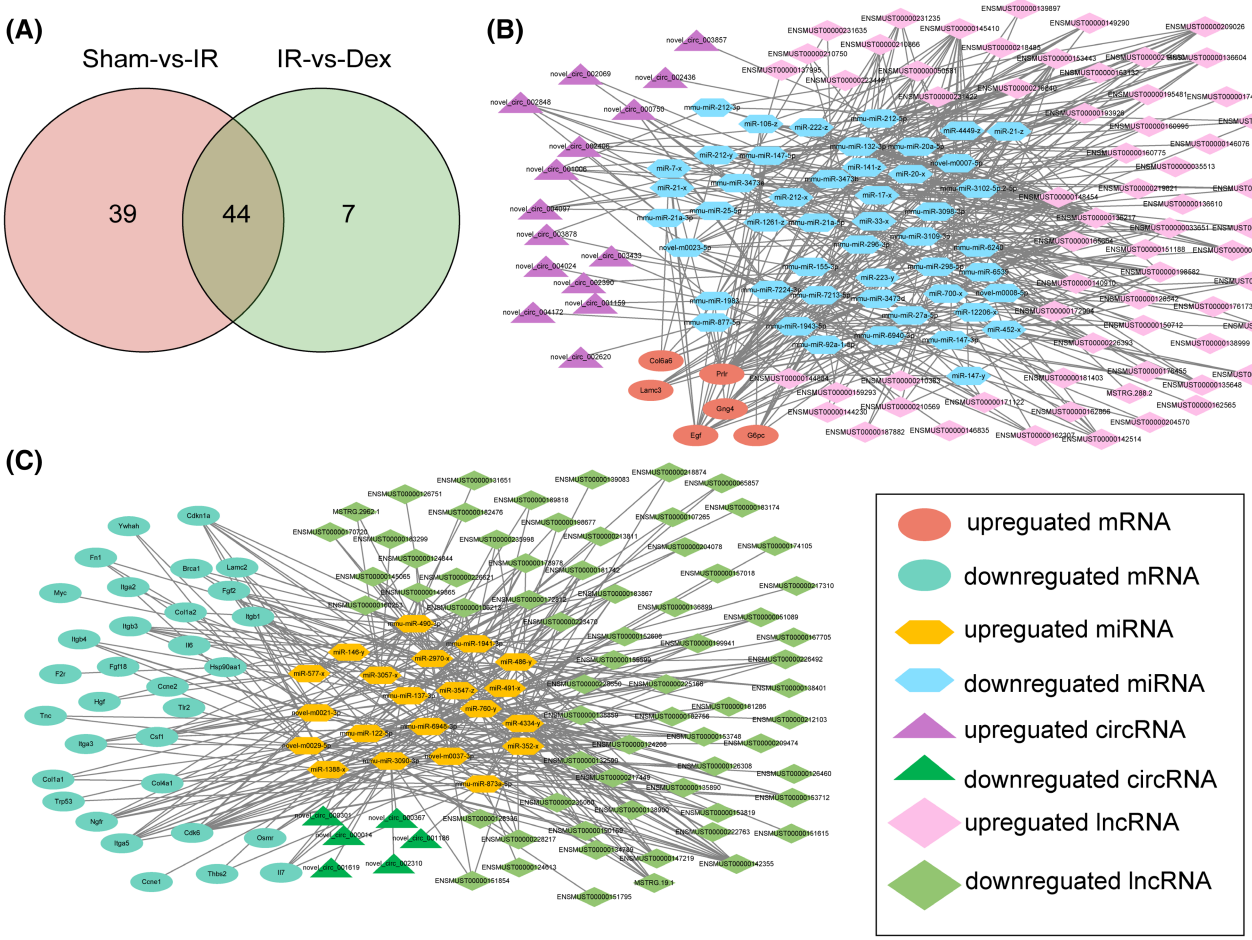

FIGURE 9 The ceRNA network associated with PI3K/Akt signaling pathway was regulated by DEX during renal I/R injury. (A) Total of 44 DEGs related to PI3K/Akt signaling pathway was regulated by DEX during renal I/R injury. (B) The ceRNA network of lncRNA/circRNA-miRNA-upregulated mRNA was regulated by DEX; (C) The ceRNA network of lncRNA/circRNA-miRNA-downregulated mRNA was regulated by DEX.

significantly improves the impaired renal function with decreasing the serum creatinine and blood urea nitrogen level. The pathophysiology of renal I/R injury is characterized by excessive activation of inflammatory responses. Especially, reperfusion followed by ischemia leads to continuous tissue damage and severe microvascular dysfunction with activating inflammation.[34] As the results of the kidney tissue from I/R-injured mice in this current study showed that I/R injury significantly increased the proinflammatory cytokines, including inflammatory factors (TNF-α and IL-6 mRNA levels), intercellular adhesion molecule (ICAM-1 mRNA level), and chemokine (MCP-1 mRNA level). Thus, activating the anti-inflammatory response is a potential strategy to improve renal I/R injury and reduce the severity of AKI. Besides, the PI3K/Akt signaling pathway, a conserved family of signal transduction enzymes, regulates inflammatory, and apoptotic events in cells undergoing noxious stimulation by the endogenous negative feedback and compensatory mechanism.[35,36] In recent years, a series of studies have found that activation of the PI3K/Akt pathway protected kidney against I/R injury through reducing apoptosis and release of inflammatory cytokines.[33,37,38] In this study, I/R significantly increased and the release of TNF-α and IL-6 resulted from the PI3K/AKT activation. And we found that DEX treatment improved the kidney damage and decreased the inflammatory response by further activating the PI3K/AKT pathway. One study indicated that DEX treatment attenuated lung I/R injury by inhibiting the apoptosis via PI3K/Akt signaling pathway.[39] Another study found that DEX prevented septic myocardial dysfunction in rats via activation of PI3K/Akt-mediated autophagy.[40] These findings suggest that the protective role of DEX on various organs I/R injury by regulating different downstream signaling by activating PI3K/AKT pathway.

The microvascular endothelial cells in kidney tissue are situated in a key position between the white blood cells and epithelial cells. In addition to regulating microvascular flow rates and permeability, the microvascular endothelial cells mediated white blood cells attachment

TABLE 2 DEGs related to PI3K/Akt signaling pathway with DEX treatment

Symbol	Log_2FC	*p*-value	FDR	ID
Up-regulated mRNAs				
Egf	4.28	<.01	<0.01	ENSMUSG00000030244
Lamc3	2.10	<.01	<0.01	ENSMUSG00000005268
G6pc	1.79	<.01	<0.01	ENSMUSG00000078650
Prlr	1.69	<.01	<0.01	ENSMUSG00000026840
Gys2	1.42	<.01	<0.01	ENSMUSG00000021303
Gng4	1.13	<.01	<0.05	ENSMUSG00000028017
Down-regulated mRNAs				
Il6	−4.84	<.01	<0.05	ENSMUSG00000025746
Spp1	−3.62	<.01	<0.01	ENSMUSG00000029304
Ccne2	−3.52	<.01	<0.01	ENSMUSG00000028212
Tnc	−3.20	<.01	<0.01	ENSMUSG00000028364
Areg	−3.08	<.01	<0.05	ENSMUSG00000029378
Ccne1	−2.74	<.01	<0.01	ENSMUSG00000002068
Thbs2	−2.29	<.01	<0.05	ENSMUSG00000023885
Hgf	−2.20	<.01	<0.01	ENSMUSG00000028864
Brca1	−2.14	<.01	<0.01	ENSMUSG00000017146
Col1a1	−1.98	<.01	<0.01	ENSMUSG00000001506
Csf1	−1.91	<.01	<0.01	ENSMUSG00000014599
Cdk6	−1.72	<.01	<0.01	ENSMUSG00000040274
Hsp90aa1	−1.71	<.01	<0.01	ENSMUSG00000021270
Il7	−1.66	<.01	<0.05	ENSMUSG00000040329
Cdkn1a	−1.66	<.01	<0.01	ENSMUSG00000023067
Fgf2	−1.65	<.01	<0.01	ENSMUSG00000037225
Osmr	−1.63	<.01	<0.01	ENSMUSG00000022146
Epha2	−1.62	<.01	<0.01	ENSMUSG00000006445
Myc	−1.62	<.01	<0.01	ENSMUSG00000022346
Ywhah	−1.53	<.01	<0.01	ENSMUSG00000018965
Fn1	−1.49	<.01	<0.01	ENSMUSG00000026193
Tlr2	−1.48	<.01	<0.05	ENSMUSG00000027995
Fgf18	−1.42	<.05	<0.05	ENSMUSG00000057967
Col1a2	−1.32	<.01	<0.01	ENSMUSG00000029661
Lamc2	−1.28	<.01	<0.01	ENSMUSG00000026479
Itgb4	−1.28	<.01	<0.01	ENSMUSG00000020758
Itgb1	−1.26	<.01	<0.01	ENSMUSG00000025809
F2r	−1.23	<.01	<0.01	ENSMUSG00000048376
Hsp90b1	−1.21	<.01	<0.01	ENSMUSG00000020048
Itga2	−1.17	<.01	<0.01	ENSMUSG00000015533
Itga3	−1.17	<.01	<0.01	ENSMUSG00000001507
Col4a1	−1.09	<.01	<0.01	ENSMUSG00000031502
Lamb3	−1.07	<.01	<0.01	ENSMUSG00000026639
Ngfr	−1.04	<.01	<0.01	ENSMUSG00000000120
Itga5	−1.03	<.01	<0.01	ENSMUSG00000000555
Trp53	−1.02	<.01	<0.01	ENSMUSG00000059552
Itgb3	−1.01	<.01	<0.05	ENSMUSG00000020689
Col6a6	1.69	<.01	<0.05	ENSMUSG00000043719

Abbreviations: DEGs, differentially expressed genes; FC, fold change; FDR, false discovery rate.

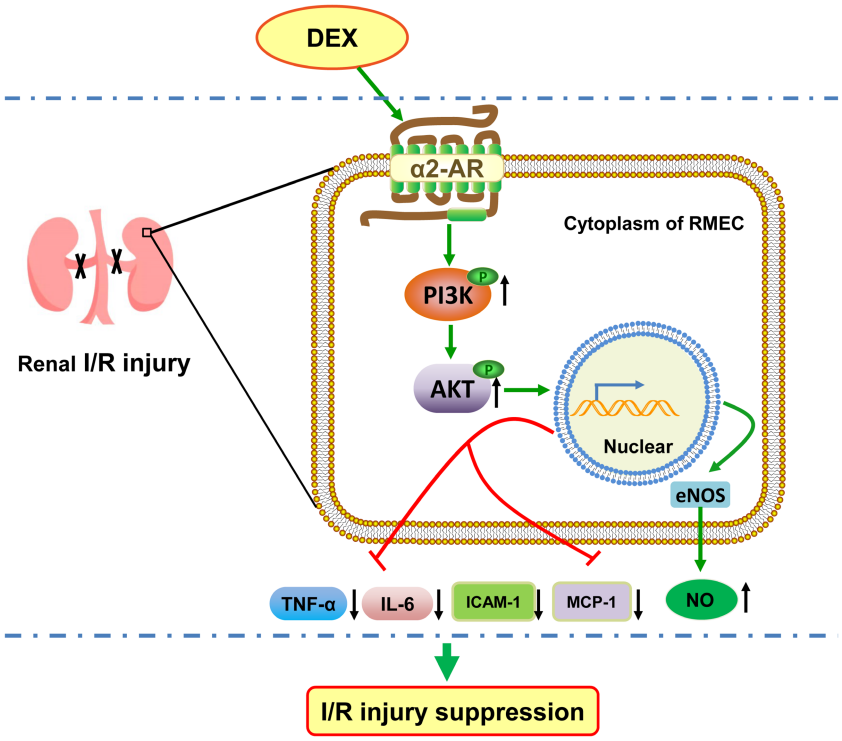

FIGURE 10 Schematic mechanism of Dex treatment-mediated protective effect against renal I/R injury through regulating renal microvascular endothelial cell. DEX treatment activated PI3K/Akt signaling by combining with the α_2-AR in RMEC during renal I/R injury. Then, PI3K/Akt signaling increased eNOS expression and decreased inflammatory factor level, which finally led to renal I/R injury suppression. α_2-AR, α_2 adrenergic receptor; DEX, dexmedetomidine; I/R, ischemia/reperfusion; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; RMEC, renal microvascular endothelial cell; TNF-α, tumor necrosis factor-α.

and migration. Although it has been demonstrated that endothelial cells play a crucial role in initiating and propagating the inflammatory response observed in ischemia, infections, and sepsis,[41] RMECs still have received less attention in the process of renal I/R injury. In our present study, RMECs have been confirmed to participant in the development and processes of renal I/R injury by the gene sequencing analysis. DEX, a highly selective α_2-AR agonist, mediated protective effect by regulating the immune system and inhibiting inflammatory responses. Besides, α_2-AR are widely expressed in the distal and proximal tubules in the kidney, as well as the peritubular vasculature.[31] Our results further indicate that α_2-AR are also rich in the cultured renal microvascular endothelial cells with using the immunofluorescence staining. And first, we demonstrate DEX treatment significantly reduces renal I/R injury by activating PI3K/Akt signaling and suppressing inflammation via α_2-AR in renal microvascular endothelial cells. Similarly, one recent study found that DEX protected cardiac microvascular endothelial cells against OGD/R-induced apoptosis via activating PPARδ-AMPK-PGC-1α signaling.[42] Another previous research indicated that DEX attenuated lung apoptosis induced by renal I/R injury through mediating the α_2AR/PI3K/Akt pathway.[43] Therefore, the microvascular endothelial cells might be the key factor for protective effects of DEX in I/R injury.

NO, a short-lived gaseous molecule synthesized from the amino acid L-arginine by the action of NO synthase (NOS), is recognized as an important mediator of physiological and pathological processes of renal I/R injury.[44] The kidney expresses three isoforms of NOS, including neuronal NOS (nNOS), inducible NOS (iNOS), and eNOS. In particular, eNOS is restricted to endothelial cells and regulated the vascular function in the kidney.[45] The role of eNOS in the progressive renal disease was detected by using eNOS knock out mice. One study found that eNOS deficiency accelerates kidney injury and the injury could be improved by L-arginine treatment.[46] And lack of eNOS resulted in greater endothelial cell injury and more severer macrophage infiltration.[47] Moreover, eNOS activation increased glomerular NO synthesis and protected against renal I/R.[48] Our present results demonstrated that DEX significantly enhanced the eNOS expression and NO levels by activating PI3K/AKT pathway. And we further focus on the role of eNOS on the response of the cultured RMECs during H/R induced injury, and found that DEX could directly improve the RMECs dysfunction through regulating PI3K/AKT-eNOS via the α_2AR. Another important finding cannot be ignored is that excessive NO production generated by the overactivated iNOS was involved in the inflammatory process and promote I/R injury.[49,50] Chatterjee et al.[49] indicated that inhibition of iNOS activity and subsequent reduction of NO level significantly improve the kidney function and reduced renal I/R injury. Thus, we suspected that NO homeostasis in the kidney tissue might be regulated by both increasing inhibiting eNOS and decreasing iNOS activity during renal I/R injury. And

more studies are still needed to verify the possible interaction between the eNOS and iNOS isoforms under renal I/R conditions.

This study has several limitations. First, the PI3K/Akt signaling pathway is also supposed to be related to cell apoptosis, but the protective role of DEX on renal I/R by decreasing the cell apoptosis via PI3K/Akt signaling was not evaluated. Second, the current results suggest that DEX-reduced renal I/R injury through activating eNOS. However, we did not detect the other NOS isoforms during I/R injury with DEX treatment. Last, the ceRNA network related to PI3K/Akt pathway has been confirmed during renal I/R injury in our results, thus the non-coding RNAs involved in renal I/R injury need to be identified in further studies.

In conclusion, this present study revealed that the protective effects of DEX against mice renal I/R injury were mediated by α_2-AR through activation of the PI3K/Akt-eNOS signaling pathway and inhibition of inflammation response in renal microvascular endothelial cells, which may provide a potential basis for the combined application of dexmedetomidine for patients at high risk of AKI in clinical therapy.

AUTHOR CONTRIBUTIONS

Fuhai Ji and Xiaowen Meng conceived the study and designed the experiments. Xisheng Shan, Jiaxin Zhang, Xiang Wei, Wenhui Tao, and Yiqing Wang performed the experiments. Hong Liu, Ke Peng, and Huayue Liu analyzed the data. Jiaxin Zhang and Xiang Wei prepared the figures. Xisheng Shan and Xiaowen Meng wrote the first draft of manuscript. Fuhai Ji revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (82072130 and 81873925 to FHJ), Natural Science Foundation of Jiangsu Province (BK20191171 to FHJ), Clinical Trial Project of Science and Technology Development Plan in Suzhou city (SLT201909 to FHJ), 333 High-level Talent Training Project in Jiangsu Province (BRA2020089 to FHJ), Six talent peaks project in Jiangsu Province (WSN-022 to FHJ), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (22KJD320002 to XWM).

DISCLOSURES

No conflict of interest exsits in the submission of this manuscript, and the manuscript is approved by all authors for publication.

DATA AVAILABILITY STATEMENT

The data used during the present study are available from the corresponding author on reasonable request. All RNA-seq processed data have been deposited in NCBI's Sequence ReadArchive (SRA) database and are accessible through accession number PRJNA766819 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA766819/).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Shan X, Zhang J, Wei X, et al. Dexmedetomidine attenuates renal ischemia–reperfusion injury through activating PI3K/Akt-eNOS signaling via α_2 adrenoreceptors in renal microvascular endothelial cells. *The FASEB Journal*. 2022;36:e22608. doi:10.1096/fj.202101626RR