

ORIGINAL ARTICLE

Inhibition of hepatocyte nuclear factor 1 β contributes to cisplatin nephrotoxicity via regulation of nf- κ b pathway

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Abstract

Cisplatin nephrotoxicity has been considered as serious side effect caused by cisplatin-based chemotherapy. Recent evidence indicates that renal tubular cell apoptosis and inflammation contribute to the progression of cisplatin-induced acute kidney injury (AKI). Hepatocyte nuclear factor 1 β (HNF1 β) has been reported to regulate the development of kidney cystogenesis, diabetic nephrotoxicity, etc. However, the regulatory mechanism of HNF1 β in cisplatin nephrotoxicity is largely unknown. In the present study, we examined the effects of HNF1 β deficiency on the development of cisplatin-induced AKI in vitro and in vivo. HNF1 β down-regulation exacerbated cisplatin-induced RPTC apoptosis by indirectly inducing NF- κ B p65 phosphorylation and nuclear translocation. HNF1 β knockdown C57BL/6 mice were constructed by injecting intravenously with HNF1 β -interfering shRNA and PEI. The HNF1 β scramble and knockdown mice were treated with 30 mg/kg cisplatin for 3 days to induce acute kidney injury. Cisplatin treatment caused increased caspase 3 cleavage and p65 phosphorylation, elevated serum urea nitrogen and creatinine, and obvious histological damage of kidney such as fractured tubules in control mice, which were enhanced in HNF1 β knockdown mice. These results suggest that HNF1 β may ameliorate cisplatin nephrotoxicity in vitro and in vivo, probably through regulating NF- κ B signalling pathway.

KEYWORDS

cisplatin nephrotoxicity, Hepatocyte nuclear factor 1 β , NF- κ B

1 | INTRODUCTION

Cisplatin is one of the most commonly used chemotherapy drugs; however, the severe side effects such as nephrotoxicity limit its dose administered in cancer patients. During cisplatin-induced acute kidney injury (AKI), renal tubular cells are extremely vulnerable to

damage because of high drug accumulation. Cisplatin nephrotoxicity is characterized by cell apoptosis and inflammation.

The nuclear factor kappa-light-chain-enhancer of activated B cell (NF- κ B) signalling pathway is important for regulating cell apoptosis. The NF- κ B p50/p65 heterodimer is mainly located in cytoplasm. Upon cisplatin stimulation and I κ B kinase activation,

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the NF- κ B translocates to nucleus and transcriptionally activates target gene expression such as interleukin-1 β (IL-1 β) and tumour necrosis factor α (TNF- α).¹ Cisplatin has the ability to induce NF- κ B phosphorylation in HEK293 cells and further decrease the secretion of pro-inflammatory cytokines such as IL-1 β and TNF- α .² Cisplatin also can increase NF- κ B expression in kidney tissues of rat.³ During cisplatin-induced mouse renal injury, NF- κ B activation, I κ B α phosphorylation and p53 protein nuclear translocation were observed. Moreover, the P53 protein level, caspase 3/9 and the poly (ADP-ribose) polymerase (PARP) cleavage were also increased.^{4,5} The transcriptional inhibition of NF- κ B was reported to be able to ameliorate cisplatin-induced AKI.⁶ However, the upstream genes regulating NF- κ B transcriptional activation during cisplatin nephrotoxicity are still not fully understood.

The transcription factor HNF1 β (hepatocyte nuclear factor 1 homeobox B) was found to be abundantly expressed in kidney and pancreas,⁷ but not in brain and heart tissues. HNF1 β was expressed higher in kidney than in lung and liver tissues.⁸ Early reports have linked HNF1 β regulation to kidney cystogenesis and development.⁹ Patients with HNF1 β mutations often complicated with diabetes, renal cysts and loss of kidney function.¹⁰⁻¹³ Upon exposure to TNF- α and interferon- γ (IFN- γ), the proximal tubular cells exhibited inhibited transcriptional activity of HNF1 β and dysfunction of mitochondria.¹⁴ Thus, the transcriptional activity of HNF1 β may be related to the inflammatory response of renal tubular cells.

HNF1 β was involved in regulating various pathological processes. HNF1 β knockdown in proximal tubular HK2 (human kidney 2) cells promoted the epithelial-to-mesenchymal transition.¹⁵ HNF1 β positively regulated the proliferation and tubulogenesis of normal kidney proximal epithelial cells (NRK-52E).¹⁶ In our previous study, HNF1 β was found to play protective role in cisplatin-induced tubular cell apoptosis *in vitro*.¹⁷ However, in Wilms' tumour-derived G401 cells, HNF1 β was reported to decrease cell proliferation and migratory abilities and increase apoptotic rate no matter through overexpression or small interfering RNA-silencing experiments.¹⁸ Therefore, the role and mechanism of HNF1 β in cisplatin-induced apoptosis still need further analysis.

In this study, the *in vivo* role of HNF1 β in cisplatin nephrotoxicity was examined. Moreover, the regulatory function of HNF1 β on NF- κ B activation was analysed.

2 | MATERIALS AND METHODS

2.1 | Regents, cell lines and animals

Rat kidney proximal tubular cells (RPTCs) were originally obtained from Dr Hopfer (Case Western Reserve University) and were cultured in DMEM/F12 medium (12400024; Gibco) containing transferrin (5 μ g/mL) (T8158; Sigma), insulin (5 μ g/mL) (I9278; Sigma), epidermal growth factor (EGF; 1 ng/mL) (E9644; Sigma), dexamethasone (4 μ g/mL) (D1756; Sigma), 10% foetal bovine serum (FBS) (PAN-Serotech) and 1% antibiotic-antimycotic (15240062;

Thermo Fisher Scientific). RPTC HNF1 β knockdown and scramble cell lines were constructed in our previous study.¹⁷ Lipofectamine 3000 Transfection Reagent (L3000008) was purchased from Thermo Fisher Scientific Company.

About 8-week-old C57BL/6J male mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (China). Mice were acclimated for 7 days before formal experiments and were housed in groups of four animals per cage. Animal experiments were performed in accordance with the approval of the Ethics Committee for Experimental Animals in Henan University (Approval Number: DWLL20200102).

2.2 | HNF1 β interference mouse model construction

The HNF1 β -specific shRNA plasmid was purchased from Qiagen Company (Catalogue Number: 336312 KR45380H, USA). The target sequence of si-HNF1 β was 5' GTGTAACAGGGCAGAATGTTT 3'. The construction of HNF1 β knockdown mice was performed as described in previous studies with mild modifications.¹⁹⁻²¹ Briefly, the HNF1 β scramble or short hairpin RNA (shRNA) was mixed with PEI (Polyethyleneimine, 765090; Sigma) at a ratio of 1:2. C57BL/6 mice aged about 8 weeks were injected with 40 μ g HNF1 β scramble or shRNA plasmids by tail vein. Mice were randomly divided into three groups: Scramble group in which mice were treated with a non-specific shRNA for 24 hours (n = 6); and shRNA-HNF1 β groups in which mice were treated with specific HNF1 β shRNA for 24 hours (n = 8) or 72 hours (n = 8).

2.3 | Cisplatin-induced AKI mouse model construction

To construct a C57BL/6 mouse model of acute kidney injury induced by cisplatin, C57BL/6 male mice aged 6-8 weeks were randomly divided into four groups: vehicle, mice treated with phosphate-buffered saline (PBS) (n = 8); cisplatin 1d, mice treated with cisplatin for 1 day (n = 8); cisplatin 2d, mice treated with cisplatin for 2 days (n = 8); and cisplatin 3d, mice treated with cisplatin for 3 days (n = 8). Mice in each group were intraperitoneally injected with 30 mg/kg cisplatin, and the cervical venous blood and kidneys of the mice were collected at 24, 48 and 72 hours after modelling, respectively.

To further evaluate the impact of HNF1 β interference on cisplatin-induced nephrotoxicity, C57BL/6 male mice aged 6-8 weeks were randomly divided into four groups as follows: shRNA-scramble group, shRNA-HNF1 β group, cisplatin with shRNA-scramble group and cisplatin with shRNA-HNF1 β group. Mice were given tail injection of 40 μ g HNF1 β -interfering plasmids. An hour later, these mice were intraperitoneally injected with PBS or 30 mg/kg cisplatin for 72 hours. The mice were anaesthetized by intraperitoneal injection of 2% chloral hydrate (0.015 mL/g; Esite Biotechnology, China). The mouse kidneys were extracted after modelling for further analysis. The mouse serum was separated from venous blood samples by

centrifugation at 12 000 g at room temperature for 5 minutes. Renal function was assessed by measuring serum creatinine (0420-500; Stanbio) and blood urea nitrogen (BUN) (0580-250; Stanbio) following Jaffe's and GLDH reactions.

2.4 | RPTC culture

The RPTC HNF1 β -negative control cells and knockdown cells were treated with 20 μ mol/L cisplatin with or without 50 μ mol/L NF- κ B pathway inhibitor TPCA (S2824; Selleck Chemicals) for 24 hours. Then, Hoechst 33342 (10 μ g/mL; Beyotime Biotechnology, China) was used for nuclear staining. The apoptotic cells were then visualized under a Nikon Inverted Microscope Eclipse Ti-E (Nikon, Japan) and counted from five randomly selected fields by ImageJ software.

2.5 | Immunofluorescence

The RPTC HNF1 β -negative control (NC) cells and knockdown (KD) cells were treated with or without 20 μ mol/L cisplatin. Briefly, 1×10^5 cells were seeded into 35-mm dishes containing slides with a diameter of 1 cm and then were cultured until 80% confluence. After 24 hours of treatment with 20 μ mol/L cisplatin, the slides were washed with PBS and penetrated with cold methanol for 10 minutes. The slides were incubated with anti-HNF1 β antibody (1:50, 12533-1-AP; Thermo Fisher Scientific) or anti-phospho-P65 antibody (1:1600, 3033; Cell Signaling Technology) overnight at 4°C and then incubated with Alexa Fluor 488-labelled secondary antibody (1:1000, Thermo Fisher Scientific, USA) for 1 hour at room temperature. Cell nucleus was stained with DAPI (P36941; Thermo Fisher Scientific) for 10 minutes. The images were acquired by Nikon Inverted Microscope Eclipse Ti-E (Nikon, Japan), and the nuclear translocation was quantified using ImageJ software as previously described.²²

2.6 | TUNEL staining

The cells with a confluence of up to 80% were extracted from the cell incubator, namely RPTC HNF-1 NC cells and RPTC HNF-1 KD cells. The cells were digested by trypsin (1004GR025, BioFroxx, Germany) and collected. The cells were evenly spread to the 24-well plate according to the density of 8×10^4 /well and then cultured for 24 hours in the cell incubator (Model: 3111; Thermo Scientific, USA) at 37°C and 5% CO₂. The next day, the cells were grouped as follows: NC or KD cells treated with or without 20 μ mol/L cisplatin, NC or KD cells treated with cisplatin and TPCA-1 (S2824; Selleck Chemicals), and NC or KD cells treated with TPCA-1 only. Cells were rinsed with PBS for 2-3 times and fixed at room temperature by 4% paraformaldehyde for 1 hour. After washed with PBS, cells were blocked with 3% H₂O₂ solution (323381; Sigma) at room temperature away from light

for 10 minutes. Cells were permeabilized with Triton X-100 (T8200; Solarbio) at concentration of 0.05% (v/v) for 2 minutes on ice and incubated with TUNEL reaction buffer (P48307791-131; Roche) at 37°C for 1 hour away from light. The cell nuclei were stained with Hoechst 33342 (10 μ g/mL) for 2 minutes at room temperature. The cells were observed under a Nikon Inverted Microscope Eclipse Ti-E (Nikon, Japan).

2.7 | Cytoplasm and nucleus protein extraction

RPTC HNF1 β NC or KD cells were treated with or without 20 μ mol/L cisplatin for 24 hours, and then, the cells were digested by SDS (S8010, Solarbio) lysis buffer and were centrifuged at 500 g for 5 minutes. The centrifuged precipitates were further used for isolation of cytoplasm and nucleus proteins by NE-PER nuclear and cytoplasmic extraction reagents (78833, Thermo Scientific).

2.8 | Western blot

RPTCs were treated with or without 20 μ mol/L cisplatin for 24 hours. Total cell proteins were extracted using SDS lysis buffer containing 1 \times Protease Inhibitor Cocktail (RF232581; Thermo) and were quantified using BCA Protein Quantification Kit (CW0014; Cwbiochem Company, China). Kidney cortex tissues were homogenized in SDS lysis buffer containing 1 \times Protease Inhibitor Cocktail at 4°C and then were centrifuged at 15 322 g for 5 minutes at 4°C. The supernatants were used for protein quantification. Protein samples were separated using SDS-PAGE (polyacrylamide gel electrophoresis) and then were transferred onto PVDF membrane (IPVH00010; Immobilon) hydrated with methanol. The membranes were incubated with primary antibodies over night at 4°C and secondary antibodies for 2 hours at room temperature. The primary antibodies were rabbit anti-HNF1 β antibody (12533-1-AP; Thermo Fisher Scientific), rabbit anti-caspase 3 antibody (9662; CST) and rabbit anti-cleaved PARP antibody (BS7047; BioWord) purchased from Cell Signaling Company. The second antibodies were HRP-labelled anti-mouse (AS003; ABdone) or anti-rabbit IgG antibody (AS014; ABdone). The ECL Chemiluminescence Detection Kit (WBKLS0500; Millipore Company) was used for signal detection. The protein bands were quantified using ImageJ software.

2.9 | Haematoxylin and eosin (H&E) staining

Kidney cortex tissue sections were placed in a 60°C oven for 4 hours, dewaxed with xylene in fume hood for 3 times, each time for 10 minutes, and then dehydrated with 100%, 96%, 90%, 80% and 70% concentrations of ethanol for 5 minutes, respectively. After washing with running tap water for 10 minutes, the sections were stained with haematoxylin for 30 seconds, decolorized with acid ethanol (1% HCl and 99% medical alcohol) for 1-2 seconds and then stained with

eosin for 40 seconds. The sections were observed and photographed under a Nikon Inverted Microscope Eclipse Ti-E (Nikon, Japan).

2.10 | Immunohistochemical staining

The dewaxing and dehydrating processes were the same as HE staining. The tissues were blocked with 3% hydrogen peroxide for 20 minutes at room temperature in the dark. Then, the sections were sealed with 5% BSA for about 30 minutes at room temperature and were incubated with primary antibodies against cleaved caspase 3 (9662; CST), HNF1 β antibody (12533-1-AP; Thermo Fisher Scientific) and phosphor-p65 (3033; Cell Signaling Technology) at dilution of 1:200 at 4°C overnight. The second antibodies were HRP-labelled anti-mouse (AS003; ABdone) or anti-rabbit IgG antibody (AS014; ABdone). The tissues were stained with 50 μ L chromogenic agent diaminobenzidine for 30 seconds and then with haematoxylin for 10 seconds. Photographs were taken under microscope with 200 \times magnification.

2.11 | Statistical analysis

The results of this experiment were all repeated for 3 times, and Prism (GraphPad Software 7) was used for data analysis. *t* Test, one-way ANOVA and two-way ANOVA were used for data comparison between groups. The data were expressed as means \pm SEM, and *P* < 0.05 was considered as statistically significant.

3 | RESULTS

3.1 | Inhibition of HNF1 β exacerbated cisplatin-induced RPTC apoptosis

We previously determined the protective role of HNF1 β in cisplatin-induced RPTC apoptosis; however, the exact mechanism of HNF1 β regulating cisplatin nephrotoxicity is still unclear. In this study, the HNF1 β -NF- κ B signalling pathway during cisplatin-induced apoptosis was examined. RPTC HNF1 β scramble (negative control, NC) and HNF1 β shRNA (knockdown, KD) cells were treated with 20 μ mol/L cisplatin for 24 hours, and total proteins were extracted for Western blot analysis. HNF1 β expression level was down-regulated to about 65.8% after stimulation of cisplatin (Figure 1A,B). Hoechst staining of RPTC nuclei indicated the cisplatin-induced apoptosis of HNF1 β knockdown cells was significantly increased in comparison with that of RPTC HNF1 β NC cells (data not shown).

3.2 | Inhibition of HNF1 β -induced NF- κ B signalling activity during cisplatin nephrotoxicity

After cisplatin treatment, the phosphorylation of p65 NF- κ B and p53 and the cleavage of caspase 3 were induced by 1.62 and 3.43 times, respectively. Compared with NC cells, cisplatin treatment of RPTCs increased p65 NF- κ B phosphorylation and caspase 3 cleavage in HNF1 β knockdown cells by 1.44 and 1.64 times, respectively (Figure 1A-E). Moreover, the cisplatin-induced NF- κ B p65 nuclear

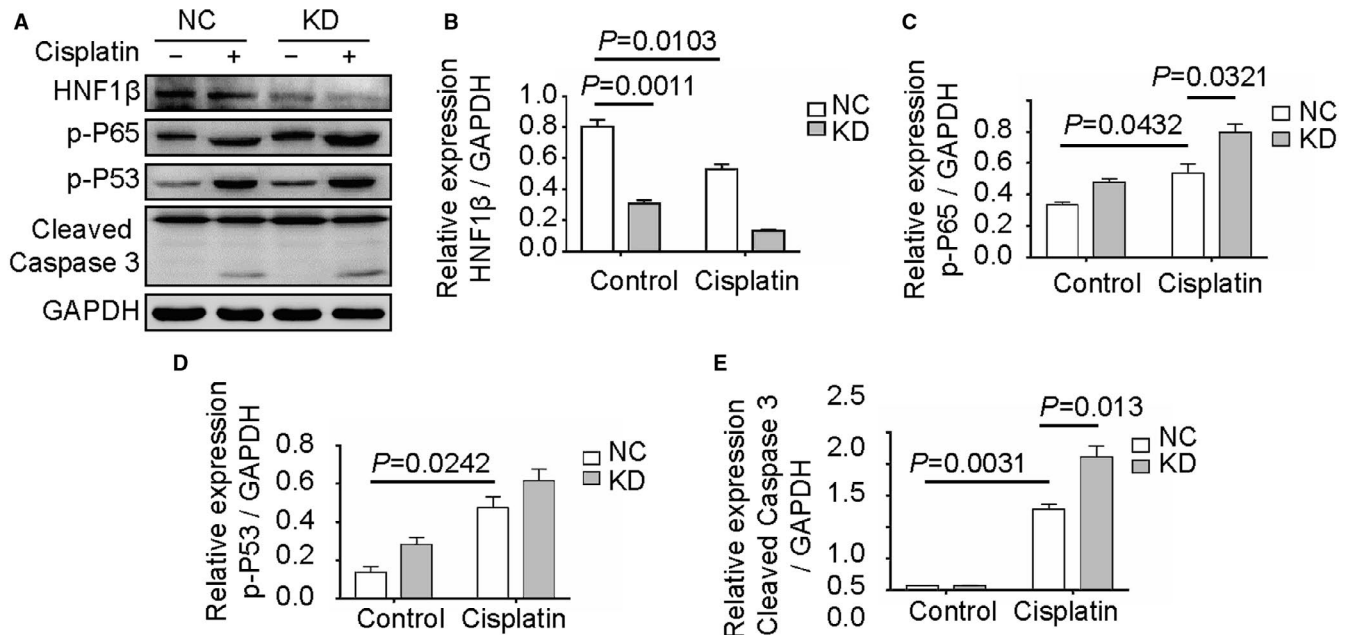


FIGURE 1 Protein expression affected by HNF1 β in RPTCs after treatment with cisplatin. RPTC HNF1 β -negative control (NC) and knockdown (KD) cells were treated with or without 20 μ mol/L cisplatin for 24 h. (A) Total proteins were extracted for Western blot analysis of HNF1 β , phosphor-P65, phosphor-P53 and cleaved caspase 3, in which GAPDH was used as an internal control. (B, C, D, E) Relative quantification and statistical analysis of HNF1 β , phosphor-P65, phosphor-P53 and cleaved caspase 3 using ImageJ software. *P* < 0.05 was considered as significant difference

translocation in RPTC HNF1 β NC and RPTC HNF1 β KD cells was examined by confocal analysis (Figure 2A). RPTCs were fixed with ethanol and stained with anti-HNF1 β antibody and FITC-labelled secondary antibody. The fluorescence intensity was quantified using

ImageJ software (Figure 2B). The green signals were mainly found in cytosol in control RPTCs. The ratio of green signal-positive cells was markedly increased after cisplatin treatment. Cisplatin promoted the nuclear translocation of P65 in RPTCs after HNF1 β interference.

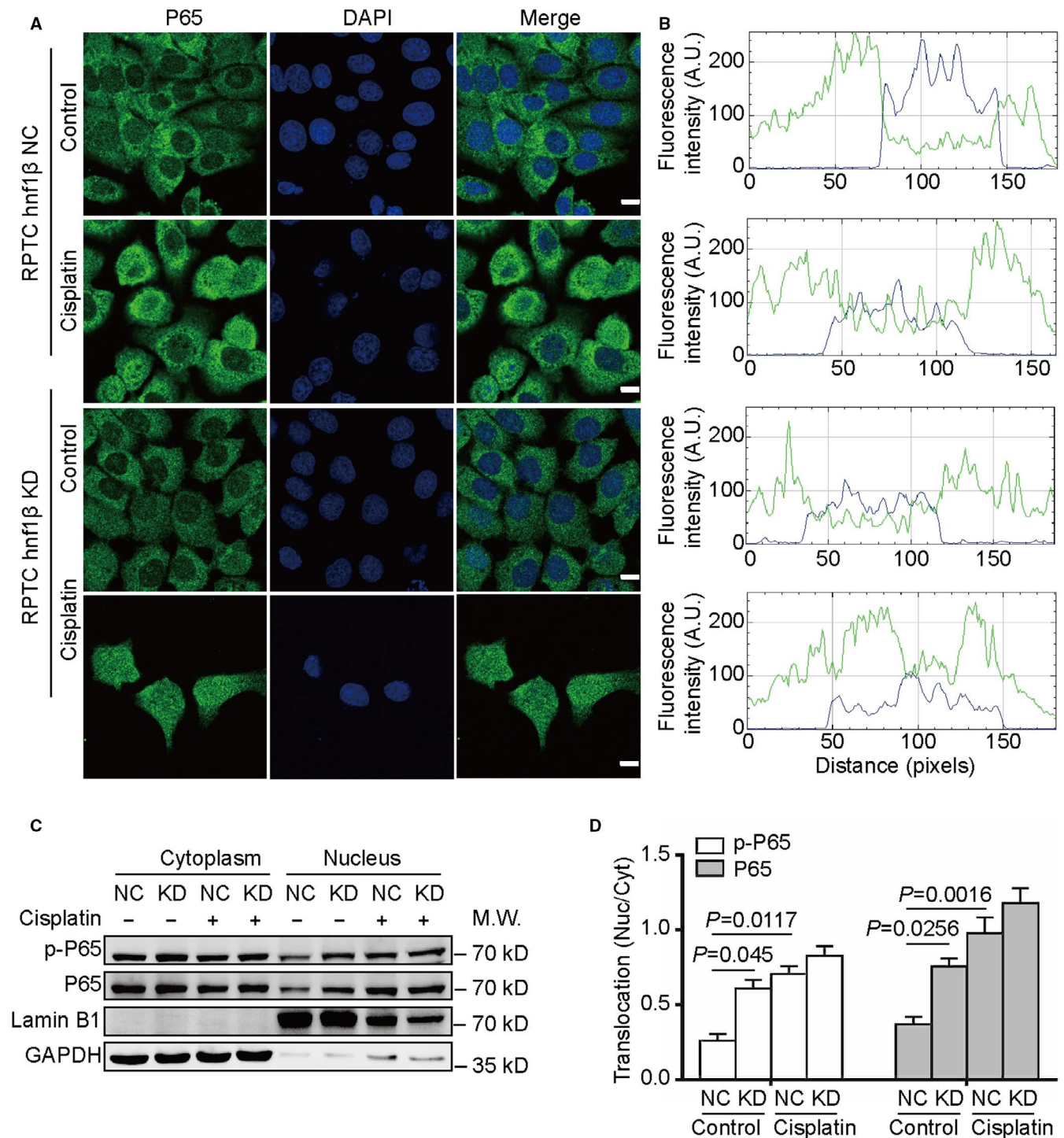


FIGURE 2 NF- κ B p65 nuclear translocation affected by HNF1 β in RPTCs after treatment with cisplatin. RPTC HNF1 β -negative control (NC) and knockdown (KD) cells were treated with or without 20 μ mol/L cisplatin for 24 h. (A) Immunofluorescence laser confocal analysis of the green (p65) and blue (nucleus) signals. Scale bars are 10 μ m. (B) Fluorescence intensities of the green and blue signals were quantified in arbitrary units (AU) by ImageJ software. (C) Western blot analysis of phosphor-P65 and total P65 in the cytoplasmic and nuclear fractions of RPTC NC and KD cells. Lamin B1 and GAPDH were used as internal controls of nuclear and cytoplasmic proteins, respectively. (D) The ratios of phosphor-P65 or total P65 in nucleus and cytoplasm were quantified using ImageJ software

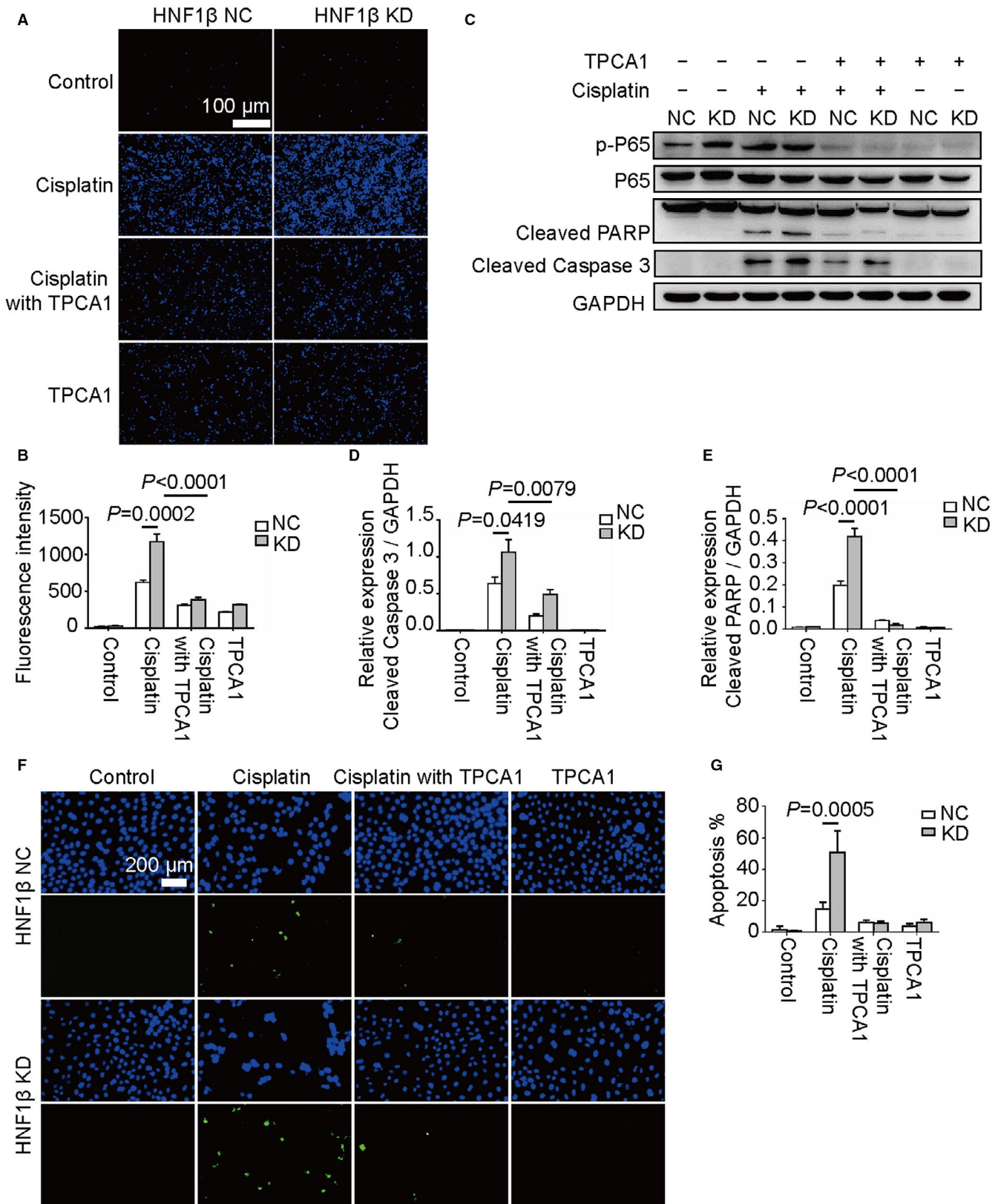
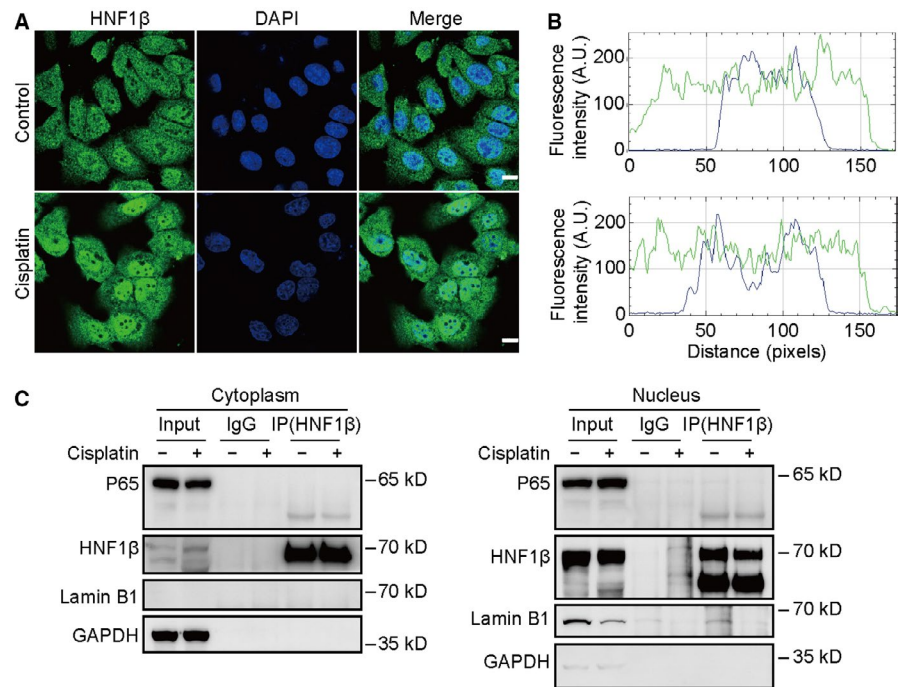


FIGURE 3 Cisplatin-induced RPTC HNF1 β NC and KD cell apoptosis affected by NF- κ B pathway inhibitor TPCA-1. The RPTC HNF1 β NC and KD cells were treated with 20 μ mol/L cisplatin in combination with 50 μ mol/L TPCA for 24 h. (A) Hoechst staining analysis of apoptotic cells. (B) The fluorescence intensities of blue signals in Figure 3A were quantified using ImageJ software. (C) Western blot analysis of phosphor-P65, total P65, cleaved PARP, cleaved caspase 3 and internal control GAPDH. (D, E) Relative quantification and statistical analysis of cleaved caspase 3 and cleaved PARP using ImageJ software. (F) TUNEL staining analysis of apoptotic cells. (G) Statistical analysis of the percentage of the number of green-positive cells in relation to the total cell number. $P < 0.05$ was considered as significant difference

FIGURE 4 HNF1 β nuclear translocation in RPTCs affected by cisplatin treatment. RPTCs were treated with or without 20 μ mol/L cisplatin for 24 h and then were immunostained with anti-HNF1 β antibody and FITC-labelled secondary antibody. The cell nucleus was stained with DAPI. (A) Fluorescence images were obtained using laser confocal microscope. Scale bars are 10 μ m. (B) The fluorescence intensities of green and blue signals in Figure 4A were quantified using ImageJ software. (C) Co-immunoprecipitation of HNF1 β and P65 proteins in cytoplasm and nucleus of RPTCs treated with or without cisplatin. Lamin B1 and GAPDH were used as internal controls of nuclear and cytoplasmic proteins, respectively



To further identify the HNF1 β -regulated nuclear translocation of p65 NF- κ B, the cytoplasm and nucleus proteins were extracted from RPTC HNF1 β NC and HNF1 β KD cells after treatment with cisplatin. The cytosolic protein GAPDH and nucleic protein lamin B1 were used as internal control for Western blot analysis (Figure 2C). The translocation of phosphor-P65 and total P65 from nucleus to cytoplasm was quantified using ImageJ software and summarized as Figure 2D. Cisplatin promoted the phosphor-P65 and total P65 translocation by 2.69 and 3.39 times, respectively. The phosphor-P65 and total P65 translocation in HNF1 β KD cells was up-regulated by 2.33 and 2.05 times, compared with HNF1 β NC cells.

3.3 | Suppression of p65 phosphorylation rescued RPTC apoptosis induced by HNF1 β down-regulation during cisplatin treatment

To further identify the impact of NF- κ B p65 signals on cisplatin-induced RPTC HNF1 β KD cell apoptosis, the p65 nuclear translocation inhibitor TPCA-1 was used for stimulation of RPTCs together with cisplatin. The apoptotic morphological changes in RPTCs were observed through the Hoechst staining assay (Figure 3A). HNF1 β interference increased the fluorescence intensity of apoptotic cells induced by cisplatin, which were inhibited by TPCA-1 (Figure 3B). In addition, Western blot analysis indicated that the TPCA-1 significantly reduced the increased PARP and caspase 3 cleavages caused by cisplatin treatment and HNF1 β knockdown, whereas only TPCA-1 treatment showed no obvious influence on RPTC apoptosis (Figure 3C). After cisplatin treatment, HNF1 β KD cells showed a 1.67-fold increase in cleaved caspase 3 and 2.13-fold increase in cleaved PARP compared with HNF1 β NC cells. HNF1 β KD cells showed a 69.3% reduction in cleaved caspase 3 and a 94.8% reduction in cleaved PARP after combined treatment of cisplatin and

TPCA-1, compared with individual treatment of cisplatin (Figure 3D,E). Moreover, TUNEL staining assay exhibited the enhanced apoptotic rate of RPTC HNF1 β KD cells compared with HNF1 β KD cells, which were all significantly reduced by TPCA-1 (Figure 3F,G).

3.4 | HNF1 β was not colocalized with NF- κ B p65 during cisplatin-induced RPTC apoptosis

As it was indicated that HNF1 β was protective against cisplatin-induced RPTC apoptosis, the nuclear translocation of HNF1 β was analysed using immunofluorescent staining. As is shown in Figure 4A, cisplatin obviously promoted colocalization of HNF1 β (green signals) and cell nucleus (blue signals). The quantification of fluorescence intensities of green and blue signals confirmed the results (Figure 4B).

To further analyse whether HNF1 β as a nuclear transcriptional regulatory factor colocalized with P65 protein, the cytoplasm and nucleus proteins of RPTCs treated with or without cisplatin were isolated and used for co-immunoprecipitation (IP) assay. To our surprise, whether or not treated with cisplatin in RPTCs, HNF1 β did not colocalize with P65 neither in cytoplasm nor in nucleus (Figure 4C).

3.5 | HNF1 β knockdown C57BL/6 mice were successfully constructed

To further analyse the function of HNF1 β in cisplatin nephrotoxicity in vivo, HNF1 β knockdown C57BL/6 mice were constructed by injecting intravenously with 40 μ g HNF1 β scramble shRNA and interfering shRNA. The kidney cortex after injection of scramble shRNA or interfering shRNA for 1 and 3 days was used for haematoxylin and eosin (H&E) and immunohistochemical staining (Figure 5A) and

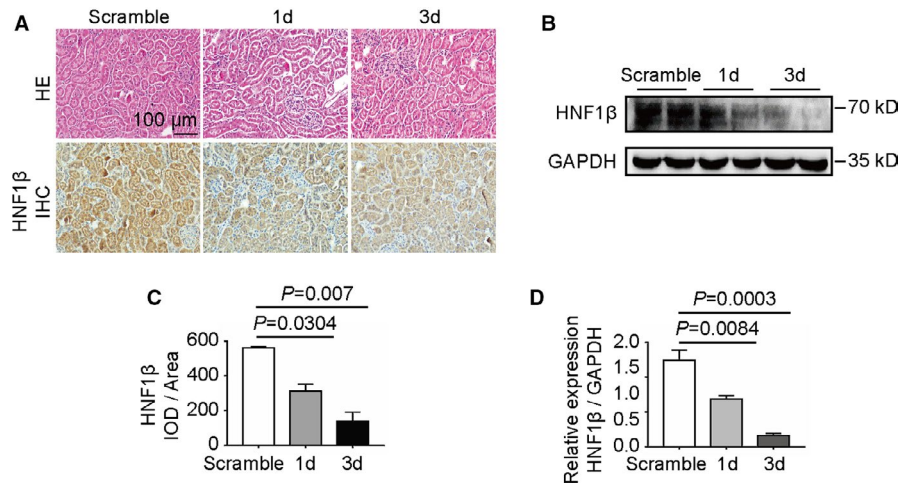


FIGURE 5 Construction of HNF1 β knockdown mice. C57BL/6 mice were injected intravenously with HNF1 β shRNA or scramble plasmids pre-mixed with PEI at a nitrogen/phosphorus weight ratio (N/P ratio) of 1:2 at room temperature for 20 min. After 3 d, the renal cortex tissues were collected. (A) HE staining and IHC staining of HNF1 β in renal cortex after interference with HNF1 β shRNA for 1 and 3 d or scramble plasmids for 3 d. (B) Western blot analysis of HNF1 β in renal cortex after interference with HNF1 β shRNA for 1 and 3 d or scramble plasmids for 3 d. (C) The mean integral optical density (IOD/area) was assessed on each section from six random fields using Image-Pro Plus 6.0 software. (D) Grey-scale analysis of the relative expression of HNF1 β protein in kidney cortex tissue in Figure 5B

Western blot analysis (Figure 5B). As shown in Figure 5A, the tubules and glomeruli of mice were relatively intact and neatly arranged after the injection of interfering plasmid for 1 and 3 days, indicating that injection of interfering plasmid has no obvious toxic effects on renal tissue. About 44.4% and 75.1% of inhibition of HNF1 β signalling were observed in renal cortex at 1 or 3 days after injection with HNF1 β knockdown plasmid compared with scramble plasmid (Figure 5C). Furthermore, Western blot assay confirmed about 44.7% and 87% reduction in HNF1 β expression in renal cortex at 1 or 3 days after injection with HNF1 β knockdown plasmid compared with scramble plasmid (Figure 5D).

3.6 | HNF1 β ameliorated cisplatin-induced nephrotoxicity in vivo

The expression of HNF1 β was further analysed in C57BL/6 mice after cisplatin treatment for 3 days. Western blot results indicated the expression level of HNF1 β was reduced after cisplatin treatment for 2 and 3 days (Figure 6A,B). The extent of caspase 3 cleavage was also enhanced after cisplatin treatment for 2 and 3 days (Figure 6C,D). The immunohistochemical staining of cleaved caspase 3 further confirmed the cisplatin-induced nephrotoxicity in day 2 and day 3 (Figure 6E,F).

To further explore the functional effect of HNF1 β , the HNF1 β scramble and knockdown mice were treated with cisplatin for 3 days to induce acute kidney injury. HE staining indicated that the tubules in the sham groups injected with scramble or HNF1 β shRNA were intact and neatly arranged. Cisplatin treatment caused obvious histological damage of kidney such as fractured tubules in control mice, which were enhanced in HNF1 β knockdown mice (Figure 7A,B).

IHC staining results indicated that cleaved caspase 3 and phosphor-p65 levels were increased in scramble mice after cisplatin treatment. Cisplatin treatment significantly increased the caspase 3 cleavage and p65 phosphorylation in HNF1 β knockdown mice, compared with control mice (Figure 7C-F). The serum urea nitrogen (BUN) and creatinine were also examined in HNF1 β scramble and knockdown mice after treatment with cisplatin. Compared with control mice, cisplatin treatment significantly increased the serum levels of BUN and creatinine by 1.39 and 1.65 times, respectively (Figure 7G,H).

4 | DISCUSSION

In this study, we found that HNF1 β protects from cisplatin-induced kidney injury both in vivo and in vitro. In addition, HNF1 β may play the protective role by negatively regulate the NF- κ B signalling pathway. Cisplatin treatment promoted the nuclear translocation of HNF1 β and p65 NF- κ B; however, no obvious interaction between these two proteins was observed in RPTCs either with or without cisplatin treatment.

It was surprising for us to observe the down-regulation of HNF1 β either in RPTCs or in C57BL/6 mice after the treatment of cisplatin. Then, we compared the HNF1 β expression levels in different time-points, and we found the HNF1 β was induced in RPTCs by cisplatin after treatment for 6-8 hours and reduced at 24 hours (data not shown). Moreover, the accumulation of HNF1 β in nucleus after cisplatin treatment was observed, indicating that the HNF1 β activation events may include nuclear translocation and regulating downstream target gene expression. HNF1 β expression was reported to be induced after ischaemia/reperfusion surgery in rat for 3-12 hours [16]. HNF1 β was up-regulated from

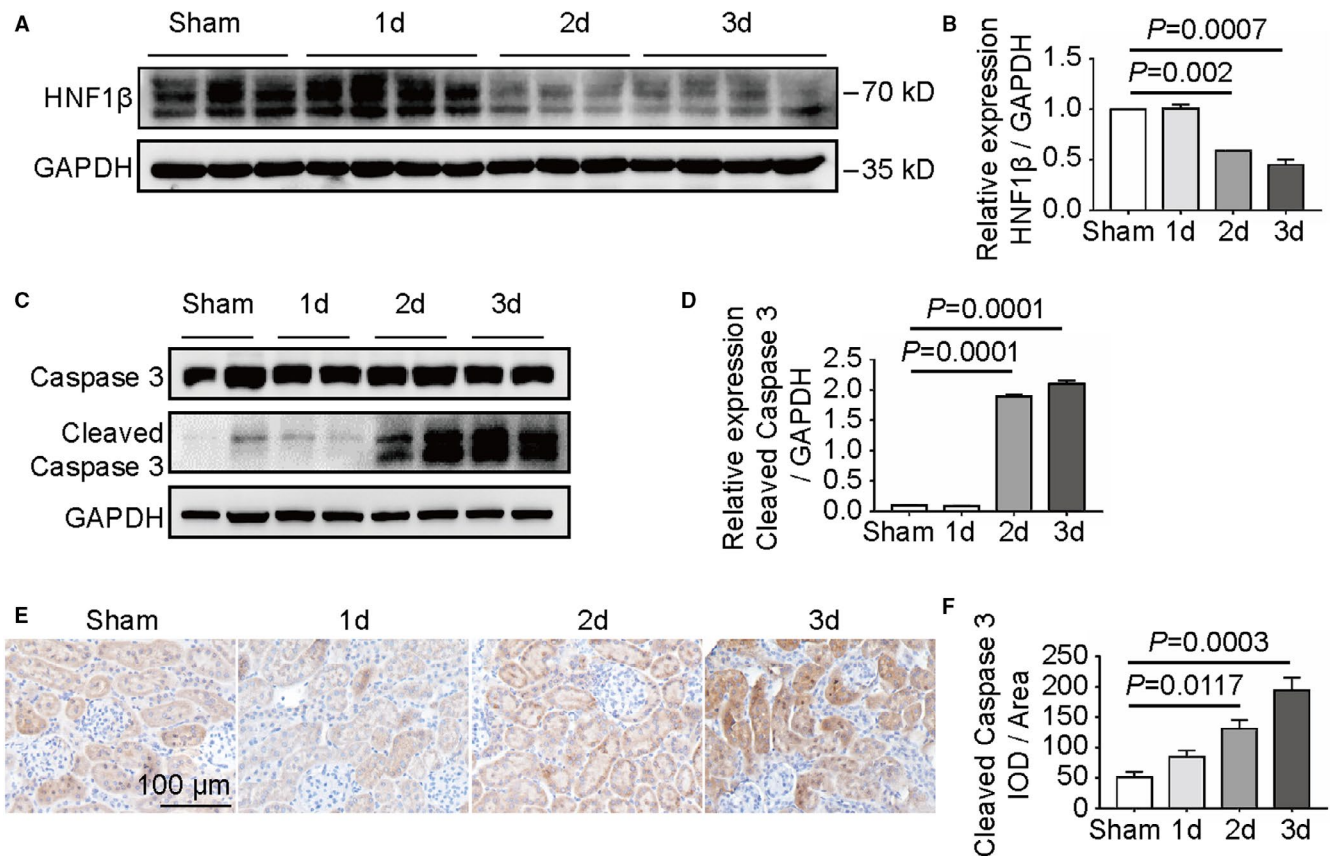


FIGURE 6 Protein expression in renal cortex tissues after treatment with cisplatin. C57BL/6 mice were injected intraperitoneally with 30 mg/kg cisplatin for 0, 1, 2 and 3 d. (A, B) Western blot analysis of HNF1 β expression and caspase 3 cleavage in renal cortex. GAPDH was used as internal loading control. (C, D) The ratios of HNF1 β and cleaved caspase 3 in relation to GAPDH in Figure 6A,B were quantified using ImageJ software. (E) IHC analysis of cleaved caspase 3 expression in renal cortex tissues. (F) The mean integral optical density (IOD/area) was assessed on each section from six random fields using Image-Pro Plus 6.0 software

1 to 24 hours after hypoxia stimulation in kidney proximal tubular HK2 (human kidney 2) cells and was down-regulated under prolonged 1% oxygen treatment [15]. The expression of HNF1 β was also down-regulated in cystic kidneys [18]. Taken together, the results demonstrated that HNF1 β expression was time-dependent and quite possible to be reduced after long-term stimulation. In addition, the transcriptional activation of HNF1 β may largely depend on the nuclear translocation.

NF- κ B activation triggers the release of cytochrome c from mitochondria, and stimulates the intrinsic apoptotic pathway, whereas P53 mainly activates extrinsic apoptotic pathway.^{23,24} Upon cisplatin stimulation, HNF1 β reduced the phosphorylation of p65 NF- κ B at serine-536 but not p53, indicating that HNF1 β may affect cisplatin nephrotoxicity through regulating the intrinsic apoptotic pathway.

HNF1 α is the other member of HNF1 transcription factor family. HNF1 α nuclear translocation was inhibited during C2-ceramide-induced hepatocyte injury.²⁵ In addition, HNF1 α increased the p65 NF- κ B expression and nuclear accumulation, leading to NF- κ B signalling activation.²⁶ However, in our study, HNF1 β nuclear translocation was enhanced after cisplatin treatment and the NF- κ B signalling was increased after HNF1 β interference, suggesting

that HNF1 β may regulate cell apoptosis in the opposite manner of HNF1 α .

Moreover, in addition to NF- κ B nuclear translocation, the NFAT5 (nuclear factor of activated T cells-5) was also reported to translocate from cytoplasm to nucleus during ultraviolet B irradiation-stimulating human lens epithelial cells. The interaction between NFAT5 and NF- κ B p65 subunit was also increased.²⁷ Thus, NFAT5 may also be the potential downstream target of HNF1 β . In this study, we did not observe the colocalization of HNF1 β and NF- κ B p65 subunit neither in cytoplasm nor in nucleus, suggesting the HNF1 β may indirectly modulate NF- κ B P65 signalling pathway.

In our previous study, blockade of NF- κ B decreased cisplatin-induced microRNA-375 expression and further increased HNF1 β activity,¹⁷ suggesting NF- κ B can negatively regulate HNF1 β activity. In this study, we confirmed that HNF1 β can influence NF- κ B signalling pathway in turn, indicating that there is a mechanism of negative feedback regulation of apoptosis pathway mediated by HNF1 β /NF- κ B.

In conclusion, the inhibition of HNF1 β induced by cisplatin contributes to nephrotoxicity either in vitro or in vivo. HNF1 β and NF- κ B signalling pathway can indirectly regulate each other and play important roles in cisplatin-induced acute kidney injury.

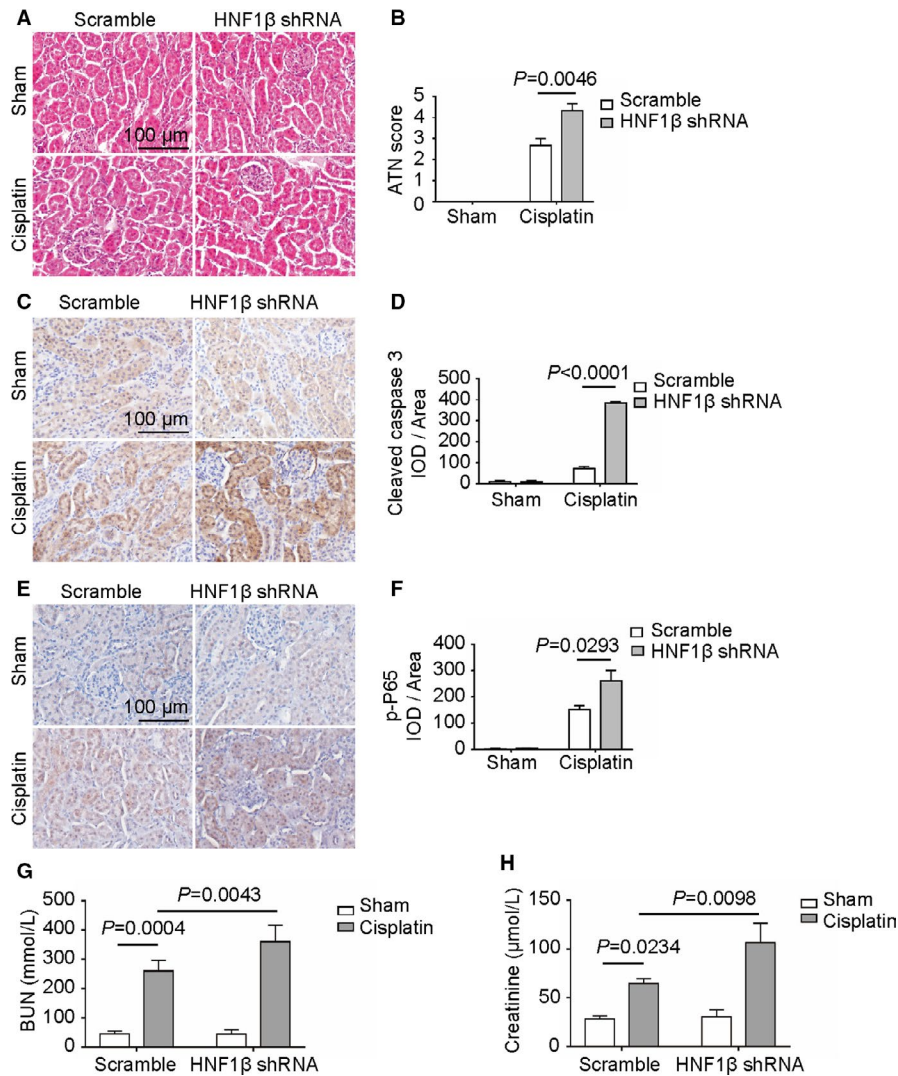


FIGURE 7 Cisplatin-induced acute kidney injury in mice with or without interference of HNF1 β . (A) HE staining of kidney cortex tissue. (B) ATN score of kidney injury shown in Figure 7A. (C) IHC staining of cleaved caspase 3 in renal cortex tissues. (D) The mean IOD/area of cleaved caspase 3 was assessed on each section from six random fields using Image-Pro Plus 6.0 software. (E) IHC staining of phosphor-P65 in renal cortex tissues. (F) The mean IOD/area of p-P65 was assessed on each section from six random fields using Image-Pro Plus 6.0 software. (G, H) The serum BUN (0580-250; STANBIO) and creatinine (0420-500; STANBIO) levels were quantified following Jaffe's and GLDH reactions

CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Yan Zhang: Formal analysis (equal); Investigation (equal); Writing-original draft (equal). **Jielu Hao:** Formal analysis (supporting); Funding acquisition (equal); Investigation (supporting). **Zijun Du:** Investigation (equal). **Peiyao Li:** Investigation (equal). **Jinghua Hu:** Writing-original draft (equal). **Mengna Ruan:** Writing-original draft (equal). **Shulian Li:** Writing-original draft (equal). **Yuanfang Ma:** Supervision (supporting). **Qiang Lou:** Conceptualization (lead); Formal analysis (equal); Funding acquisition (lead); Investigation (equal); Project administration (lead); Supervision (lead); Writing-review & editing (lead).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Hao J, Li Z, Zhang C, et al. Targeting NF-kappaB/AP-2beta signaling to enhance antitumor activity of cisplatin by melatonin in hepatocellular carcinoma cells. *Am J Cancer Res*. 2017;7(1):13-27.
- Zhou YD, Hou JG, Yang G, et al. Icariin ameliorates cisplatin-induced cytotoxicity in human embryonic kidney 293 cells by suppressing ROS-mediated PI3K/Akt pathway. *Biomed Pharmacother*. 2019;109:2309-2317. <https://doi.org/10.1016/j.biopha.2018.11.108>
- Mahrn YF. New insights into the protection of growth hormone in cisplatin-induced nephrotoxicity: the impact of IGF-1 on the Keap1-Nrf2/HO-1 signaling. *Life Sci*. 2020;253:117581. <https://doi.org/10.1016/j.lfs.2020.117581>
- Wang Z, Sun W, Sun X, Wang Y, Zhou M. Kaempferol ameliorates Cisplatin induced nephrotoxicity by modulating oxidative stress, inflammation and apoptosis via ERK and NF-kappaB pathways. *AMB Express*. 2020;10(1):58. <https://doi.org/10.1186/s13568-020-00993-w>
- Jin F, Chen X, Yan H, et al. Bisdemethoxycurcumin attenuates cisplatin-induced renal injury through anti-apoptosis, anti-oxidant and anti-inflammatory. *Eur J Pharmacol*. 2020;874:173026. <https://doi.org/10.1016/j.ejphar.2020.173026>
- Ozkok A, Ravichandran K, Wang Q, Ljubanovic D, Edelstein CL. NF-kB transcriptional inhibition ameliorates cisplatin-induced acute

- kidney injury (AKI). *Toxicol Lett.* 2016;240(1):105-113. <https://doi.org/10.1016/j.toxlet.2015.10.028>
7. Gong HY, Lin CJ, Chen MH, et al. Two distinct teleost hepatocyte nuclear factor 1 genes, *hnf1alpha/tcf1* and *hnf1beta/tcf2*, abundantly expressed in liver, pancreas, gut and kidney of zebrafish. *Gene.* 2004;338(1):35-46. <https://doi.org/10.1016/j.gene.2004.05.003>
 8. Liu Y, Kanyomse Q, Xie Y. Tumor-suppressive activity of *Hnf1beta* in Wilms' tumor. *Biosci Biotechnol Biochem.* 2019;83(11):2008-2015. <https://doi.org/10.1080/09168451.2019.1611409>
 9. Singh V, Singla SK, Jha V, Puri V, Puri S. Hepatocyte nuclear factor-1beta: a regulator of kidney development and cystogenesis. *Indian J Nephrol.* 2015;25(2):70-76. <https://doi.org/10.4103/0971-4065.139492>
 10. Verhave JC, Bech AP, Wetzels JF, Nijenhuis T. Hepatocyte nuclear factor 1beta-associated kidney disease: more than renal cysts and diabetes. *J Am Soc Nephrol.* 2016;27(2):345-353. <https://doi.org/10.1681/ASN.2015050544>
 11. Clissold RL, Hamilton AJ, Hattersley AT, Ellard S, Bingham C. HNF1B-associated renal and extra-renal disease-an expanding clinical spectrum. *Nat Rev Nephrol.* 2015;11(2):102-112. <https://doi.org/10.1038/nrneph.2014.232>
 12. Okorn C, Goertz A, Vester U, et al. HNF1B nephropathy has a slow-progressive phenotype in childhood-with the exception of very early onset cases: results of the German Multicenter HNF1B Childhood Registry. *Pediatr Nephrol.* 2019;34(6):1065-1075. <https://doi.org/10.1007/s00467-018-4188-8>
 13. Urakami T. Maturity-onset diabetes of the young (MODY): current perspectives on diagnosis and treatment. *Diabetes Metab Syndr Obes.* 2019;12:1047-1056. <https://doi.org/10.2147/DMSO.S179793>
 14. Casemayou A, Fournel A, Bagattin A, et al. Hepatocyte nuclear factor-1beta controls mitochondrial respiration in renal tubular cells. *J Am Soc Nephrol.* 2017;28(11):3205-3217. <https://doi.org/10.1681/ASN.2016050508>
 15. Faguer S, Mayeur N, Casemayou A, et al. *Hnf-1beta* transcription factor is an early *hif-1alpha*-independent marker of epithelial hypoxia and controls renal repair. *PLoS One.* 2013;8(5):e63585. <https://doi.org/10.1371/journal.pone.0063585>
 16. Ogata K, Shimamura Y, Hamada K, et al. Upregulation of HNF-1beta during experimental acute kidney injury plays a crucial role in renal tubule regeneration. *Am J Physiol Renal Physiol.* 2012;303(5):F689-F699. <https://doi.org/10.1152/ajprenal.00086.2012>
 17. Hao J, Lou Q, Wei Q, et al. MicroRNA-375 is induced in cisplatin nephrotoxicity to repress hepatocyte nuclear factor 1-beta. *J Biol Chem.* 2017;292(11):4571-4582. <https://doi.org/10.1074/jbc.M116.754929>
 18. Shao A, Chan SC, Igarashi P. Role of transcription factor hepatocyte nuclear factor-1beta in polycystic kidney disease. *Cell Signal.* 2020;71:109568. <https://doi.org/10.1016/j.cellsig.2020.109568>
 19. Narvaez A, Guiteras R, Sola A, et al. siRNA-silencing of CD40 attenuates unilateral ureteral obstruction-induced kidney injury in mice. *PLoS One.* 2019;14(4):e0215232. <https://doi.org/10.1371/journal.pone.0215232>
 20. Hong YA, Jung SY, Yang KJ, et al. Cilastatin preconditioning attenuates renal ischemia-reperfusion injury via hypoxia inducible factor-1alpha activation. *Int J Mol Sci.* 2020;21(10):3583. <https://doi.org/10.3390/ijms21103583>
 21. Huang G, Bao J, Shao X, et al. Inhibiting pannexin-1 alleviates sepsis-induced acute kidney injury via decreasing NLRP3 inflammasome activation and cell apoptosis. *Life Sci.* 2020;254:117791. <https://doi.org/10.1016/j.lfs.2020.117791>
 22. Noursadeghi M, Tsang J, Haustein T, Miller RF, Chain BM, Katz DR. Quantitative imaging assay for NF-kappaB nuclear translocation in primary human macrophages. *J Immunol Methods.* 2008;329(1-2):194-200. <https://doi.org/10.1016/j.jim.2007.10.015>
 23. Albensi BC. What is nuclear factor kappa B (NF-kappaB) doing in and to the mitochondrion? *Front Cell Dev Biol.* 2019;7:154. <https://doi.org/10.3389/fcell.2019.00154>
 24. Ji Y, Shen J, Li M, et al. RMP/URI inhibits both intrinsic and extrinsic apoptosis through different signaling pathways. *Int J Biol Sci.* 2019;15(12):2692-2706. <https://doi.org/10.7150/ijbs.36829>
 25. Li Y, Hao B, Muhammad I, et al. Acetaminophen-induced reduction in glutathione-S-transferase A1 in hepatocytes: a role for hepatic nuclear factor 1alpha and its response element. *Biochem Biophys Res Commun.* 2019;516(1):251-257. <https://doi.org/10.1016/j.bbrc.2019.06.019>
 26. Lin J, Gu C, Shen Z, et al. Hepatocyte nuclear factor 1alpha down-regulates HBV gene expression and replication by activating the NF-kappaB signaling pathway. *PLoS One.* 2017;12(3):e0174017. <https://doi.org/10.1371/journal.pone.0174017>
 27. Chung I, Hah YS, Ju S, et al. Ultraviolet B Radiation Stimulates the Interaction between Nuclear Factor of Activated T Cells 5 (NFAT5) and Nuclear Factor-Kappa B (NF-kappaB) in human lens epithelial cells. *Curr Eye Res.* 2017;42(7):987-994. <https://doi.org/10.1080/02713683.2016.1270327>

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