P53 Contributes to Cisplatin Induced Renal Oxidative Damage via Regulating P66shc and MnSOD

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p53  •  MnSOD  •  p66shc  •  Cisplatin nephrotoxicity

Abstract
Background/Aims: Cisplatin is widely used to treat malignancies. However, its major limitation is the development of dose-dependent nephrotoxicity. The precise mechanisms of cisplatin-induced kidney damage remain unclear. Previous study demonstrated the central role of mitochondrial ROS (mtROS) in the pathogenesis of cisplatin nephrotoxicity. The purpose of this study was to explore the mechanism of mtROS regulation in cisplatin nephrotoxicity. Methods: p53, MnSOD and p66shc were detected at mRNA and protein levels by qPCR and western blot in HK2 cells. mtROS levels were determined by DCFDA and MitoSOX staining. Cell viability and cell apoptosis were accessed by CCK-8 assay, TUNEL assay and flow cytometry, respectively. siRNAs were used to knock down p53 and p66shc expression and subsequent changes were observed. In vivo assays using a mouse model of cisplatin-induced acute kidney injury were used to validate the in vitro results. Results: In HK2 cells, cisplatin exposure decreased the MnSOD and increased the expression of p53 and p66shc. MnTBAP, a MnSOD mimic, blocked cisplatin-induced the generation of mtROS and cell injury. P66shc and p53 siRNAs rendered renal cells resistant to cisplatin-induced mtROS production and cell death. Furthermore, knockdown of p53 restored MnSOD and inhibiting p66shc. Consistent with these results, we revealed that p53 inhibitor reduced cisplatin-induced oxidative stress and apoptosis by regulating MnSOD and p66shc in the kidney of cisplatin-treated mice. Conclusion: Our study identifies activation of p53 signalling as a potential strategy for reducing the nephrotoxicity associated with cisplatin treatments and, as a result, broadens the therapeutic window of this chemotherapeutic agent.

Y. Yuan and H. Wang contributed equally to this work.
Introduction

Cisplatin is an antitumor drug widely used in the treatment of many malignant tumours [1, 2]. However, the drug’s use is limited because of its side effects in normal tissues, mainly injury to the kidneys [3, 4]. The clinical characteristics of cisplatin-induced nephrotoxicity are renal wasting of sodium, calcium, magnesium and amino acids, and decreased glomerular filtration rate [5]. The main site of cisplatin action is in proximal tubule epithelial cells [6]. Although reactive oxygen species (ROS) are regarded as a central key in cisplatin-induced nephrotoxicity [7], the exact roles of free radicals and the mechanisms involved in cisplatin cytotoxicity in renal tubule cells are still not completely understood.

Mitochondria, as the sites of aerobic respiration, are the principal generators of ROS in the cell [8]. Several lines of evidence demonstrated that cisplatin accumulated in the mitochondria of renal cells, hampering mitochondrial bioenergetics, increasing the production of ROS, inhibiting the absorption of calcium in the mitochondria, and causing the release of pro-apoptotic factors which ultimately result in renal tubular cell death [9]. Mitochondrial ROS (mtROS) production is tightly regulated by a number of factors, such as mitochondrial membrane potential, metabolic state of mitochondria, and O₂ levels [10].

Materials and Methods

Reagents and antibodies

Cisplatin, N-acetyl-cysteine (NAC), apocynin and Pifithrin-α were purchased from Sigma (St. Louis, MO). MnTBAP was from Santa Cruz (Santa Cruz, CA). The plasmid for p66shc S36A constructs was obtained from Addgene (Cambridge, MA, USA). The following primary antibodies were used: anti-p53 and anti-MnSOD from Santa Cruz (Santa Cruz, CA), anti-p66shc, anti-Bax, anti-p21, anti-Phospho-p53 (Ser15), anti-acetylated-Lys382 p53, anti-Mdm2 and anti-β-actin from Cell Signaling (Danvers, MA), anti-phosphorylated foxo3a and anti-catalase antibodies from Abcam (Cambridge, MA, USA). All secondary antibodies for
immunoblot analysis were from Cell Signaling (Danvers, MA). MitoSOX and DCFDA were from Invitrogen (Carlsbad, CA, USA).

**Cell culture**
HK2 cells, an immortalized proximal tubular epithelial cell line from human, were grown in DMEM media supplemented with 10% FBS and antibiotics (100 U/ml penicillin G, 100 µg/ml streptomycin). The cells were grown at 37°C with 5% CO2 and subcultured at 50–80% confluence using 0.05% trypsin-0.02% EDTA (Invitrogen).

**Experimental models of acute kidney injury**
Study protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Nanjing Medical University, China. In brief, male mice of 8-10 weeks old were used for experiments. For cisplatin injury, mice were intraperitoneally injected with a single dose of cisplatin at 20mg/kg. Control animals were injected with a comparable volume of saline. To test the effect of Pifithrin-α, 2.2mg/kg Pifithrin-α was injected (i.p.) 1 hour prior to cisplatin administration and then daily after cisplatin treatment. All quantitative data were obtained from six mice per group with each mouse coming from a separate experiment.

**Quantitative PCR**
Total RNA was isolated from cells or kidney tissues from cortex extract using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. Reverse transcription was performed using a reaction kit (Promega Reverse Transcriptase System) according to the manufacture’s protocol. Real-time PCR amplification was performed using the ABI 7500 Real-time PCR Detection System (Foster City, CA, USA) with the SYBR Green master mix (Applied Biosystems). Primers used were as follows: human p53, 5’-GTT CCG AGA GCT GAA TGA GG-3’ and 5’-TGA TGG CGG GAG GTA GAC TG-3’; human p66shc, 5’-CCA CTC CGG AAT GAG TCT CT-3’ and 5’-GAA GGA GCA CAG GGT AGT GG-3’; human MnSOD, 5’-ACA GGC CTT ATT CCA CTG CT-3’ and 5’-CAG CAT AAC GAT CCT GGT TT-3’; human GAPDH 5’-AGG GCT GCT TTT AAC TCT GGT-3’ and 5’-GCC CAC TTG ATT TTG GAG GGA-3’. Relative mRNA expression was quantified using the ΔΔCt method and GAPDH was used as an internal control. Results were expressed as fold change.

**Western blots**
Thirty micrograms of proteins from cells or kidney tissues from cortex extract were separated by acrylamide electrophoresis. Proteins were transferred to nitrocellulose membranes by electrophotting and incubated with antibodies, followed by horseradish peroxidase-conjugated secondary antibodies. The blots were visualized with Amersham™ ECL™ Detection Systems (Amersham). Densitometric analysis was performed using Quantity One Software (Bio-Rad, Hercules, CA, USA).

**Transient transfection**
HK-2 cells were cultivated to 50–60% confluence in culture medium containing no penicillin or streptomycin. Transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol.

**RNA interference**
Predesigned siRNA to human p53, p66shc and control scrambled siRNA were purchased from Santa Cruz Biotechnology (USA). HK-2 cells were transiently transfected with 100 nM siRNA constructs using X-tremeGENE siRNA transfection reagent (Roche Applied Science, Germany) according to the manufacturer’s protocol. Efficiency of knockdown was performed through real-time PCR and Western blot analysis.

**Assessment of ROS levels**
Mammalian cells were plated on a 6 well plate the day before the experiment. Following exposure to the drug, superoxide levels were assessed by incubating cells with MitoSOX (5 µM; Molecular Probes) for 30 min at 37°C. Following incubation with fluorescent probes, cells were washed twice resuspended in PBS and assessed for fluorescence intensity by flow cytometry. H2O2 production and distribution were measured. In brief, after drug exposure, the cells were double-stained by DCFDA (10 µM; Molecular Probes).
and MitoTracker Red (25 nM; Molecular Probes) for 30 min in the dark at 37°C. Both the DCF and mitotracker fluorescence were visualized under the Zeiss LSM. The fluorescence intensity of DCF was measured by flow cytometry.

**Cell viability and cell apoptosis**

The cell viability was determined by CCK-8 assay according to the manufacturer’s protocol as previously reported [16]. Apoptosis in cells and kidney tissues were analyzed by TUNEL assay using the In Situ Cell Death Detection Kit from Roche Applied Science, as described previously [17]. *In vitro*, apoptosis was quantified by flow cytometry using annexin V-fluorescein isothiocyanate and propidium iodide double staining (annexin V-fluorescein isothiocyanate apoptosis detection kit, BD Biosciences, San Diego, CA), according to the manufacturer’s instructions. For quantification *in vivo*, 10 fields were randomly selected from each tissue section to count the TUNEL-positive cells per millimeter.

**Renal function and histology**

To monitor renal function, serum creatinine and blood urea nitrogen (BUN) levels were determined using commercial kits. Creatinine was measured with a commercial kit from BioAssay Systems (Hayward, CA, USA). BUN was measured with a kit from Jiancheng Bioengineering Institute (Nanjing, China). For histology, kidney tissues were fixed with 4% paraformaldehyde for paraffin embedding and stained with periodic acid–Schiff (PAS) reagent. Tubular injury was assessed in PAS sections using a semiquantitative scale [18]. Histological changes due to tubular necrosis were quantitated by counting the percent of tubules with evident cell lysis, loss of brush border, tubule dilation, and cast formations as follows: 0 = normal, 1 = <10%, 2 = 10–25%, 3 = 26–75%, 4 = >75%. At least 5–10 fields (×200) were reviewed for each slide.

**Measurement of malondialdehyde (MDA) concentration**

Levels of MDA content in kidney cortex were assessed in the form of thiobarbituric acid-reactive substances as previously described [19]. Results are expressed as nanomoles per milligram of protein.

**Statistical analysis**

Data are expressed as the average ± SEM of the indicated number of experiments. Statistical analysis was performed with the SPSS 13.0 software using the one-way ANOVA analysis followed by Bonferroni correction. A statistical value of *P* < 0.05 was considered significant.

**Results**

**Effect of cisplatin on the expression of p53, p66shc and MnSOD in HK-2 cells**

To determine whether p53, p66shc and MnSOD play a role in cisplatin-induced cell injury, we examined their expression in HK2 cells after treatment with different doses of cisplatin. As expected, RT-PCR and western blot analysis showed that cisplatin significantly induced p53 and p66shc expression in a dose-dependent manner. Conversely, MnSOD expression was repressed after treatment with three different doses of cisplatin (Fig. 1A and 1B). Consistently, a time course study showed that cisplatin increased p53 and p66shc expression and decreased MnSOD expression (Fig. 1C and 1D).

**Effect of cisplatin on post-translational modifications of p53, the expressions of p53 downstream genes and mtROS**

In response to different cellular signals, p53 is activated by covalent modifications, including phosphorylation, acetylation, ubiquitylation, neddylation, sumoylation, and methylation. These post-translational modifications of p53 play an essential role in both stabilization and activation of the protein [20]. As shown in Fig. 2A, cisplatin increased the phosphorylation and acetylation levels of p53 after 6 h of treatment. In addition, cisplatin induced the expression of mouse double minute protein 2 (Mdm2), which acts as a key negative regulator of p53 activity through its ability to facilitate p53 degradation via the ubiquitin-proteasome pathway. Meanwhile, the expression of other p53 target genes,
NAC abrogated the cisplatin-induced ROS production, which indicated that cisplatin-induced ROS accumulation (Fig. 3C). The same protective effect against the accumulation of \( \text{H}_2\text{O}_2 \) was observed in HK2 cells pretreated with MnTBAP (Fig. 3C and 3D). CCK-8 assay revealed that treatment with MnTBAP significantly decreased cisplatin-induced cytotoxicity in HK2 cells (Fig. 3E). Also, pretreatment with MnTBAP notably prevented against cisplatin-induced apoptosis in HK2 cells (Fig. 3F and 3G).

**Fig. 1.** Effect of cisplatin on the expression of MnSOD, p66shc and p53 in HK2 cells. (A) Confluent HK-2 cells were incubated with cisplatin at the indicated concentrations for 12 h, expression levels of the indicated genes were determined by real-time RT-PCR. Gene expression levels are expressed relative to levels in control group. (B) HK-2 cells were treated with cisplatin at indicated doses for 24 h. MnSOD, p66shc and p53 protein expressions were detected by immunoblotting. Top: representative immunoblots. Bottom: densitometric analysis. (C, D) Time course of MnSOD, p66shc and p53 expressions treated with cisplatin. Confluent HK-2 cells were incubated with 25 \( \mu \text{M} \) cisplatin for the indicated periods of time. MnSOD, p66shc and p53 expressions were detected by real-time RT-PCR (C) and immunoblotting (D) respectively. Top: representative immunoblots. Bottom: densitometric analysis. Values are means \( \pm \) SEM from three independent experiments. *\( P < 0.01 \) versus control group. Three separate experiments were performed with comparable results.

including p21 and Bax, was significantly induced in HK2 cells 12 h after cisplatin treatment. Then, we characterized the origin of ROS after cisplatin treatment using the nicotinamide (NAM) adenine dinucleotide phosphate oxidase inhibitor apocynin, MnSOD mimic MnTBAP and a widely used antioxidant NAC, respectively. As shown in Fig. 2B, apocynin, MnTBAP and NAC abrogated the cisplatin-induced ROS production, which indicated that cisplatin-induced ROS production originated from both the mitochondria and NADPH oxidase.

**MnTBAP blocked cisplatin-induced mtROS and cytotoxicity in HK2 cells**

Given that MnSOD is an important mitochondrial oxidation scavenger and its expression progressively declined after cisplatin treatment, we tested the role of MnSOD in mtROS and cell damage by using MnTBAP. As shown in Fig. 3A and 3B, MnTBAP almost blocked cisplatin-induced mtROS production. Colocalization experiments between DCFDA and MitoTracker Red signals further confirmed that the mitochondria was the major site of cisplatin-induced ROS accumulation (Fig. 3C). The same protective effect against the accumulation of \( \text{H}_2\text{O}_2 \) was observed in HK2 cells pretreated with MnTBAP (Fig. 3C and 3D). CCK-8 assay revealed that treatment with MnTBAP significantly decreased cisplatin-induced cytotoxicity in HK2 cells (Fig. 3E). Also, pretreatment with MnTBAP notably prevented against cisplatin-induced apoptosis in HK2 cells (Fig. 3F and 3G).
Suppression of p66shc inhibited cisplatin-induced mtROS and cytotoxicity in HK-2 cells

Previous study showed that p66shc induced ROS production via inhibiting foxo3a activity [15]. Foxo3a is a key transcription factor that translocates to nucleus and activates transcription by specifically binding to the promoters of target genes, causing an activation of transcription of the two essential antioxidant enzymes MnSOD and catalase, which scavenge superoxide and hydrogen peroxide, respectively [21]. P66shc expression was inhibited (~60% reduction) by p66Shc siRNA (Fig. 4A and 4B). Cisplatin reduced Foxo3a phosphorylation and catalase expression, and p66shc siRNA abolished the effects of cisplatin (Fig. 4C). These data suggested that p66shc-foxo3a-catalase signalling might play an important role in modulating ROS accumulation. Then we tested the possibility that p66shc might mediate cell injury via mtROS production in our experimental model. As shown in Fig 4D, p66shc mutant inhibited cisplatin-induced mtROS production. Moreover, cisplatin-induced mtROS generation was abolished following transfection with p66shc siRNA (Fig. 5A and 5B). Also, p66shc siRNA inhibited cisplatin-induced H₂O₂ production (Fig. 5C and 5D). Down regulation of p66shc by siRNA reduced cisplatin-induced injury to HK2 cells assessed by cell viability (Fig. 5E) and cell apoptosis (Fig. 5F and 5G). These data indicated that cisplatin induced p66shc expression, and it conceivably mediated mtROS and cell injury.

Suppression of p53 restored MnSOD expression and inhibited p66shc expression in HK-2 cells

We next examined the specific functional role of p53 on the regulation of MnSOD and p66shc. P53 expression was inhibited by p53 siRNA after transfection (~70%) (Fig. 6A and 6B). As shown in Fig. 6C and 6D, the defect in scavenging capacity and the excess in mtROS...
Suppression of p53 inhibited cisplatin-induced mtROS and cytotoxicity in HK-2 cells

The dependency of p53 in cisplatin-induced mtROS and cytotoxicity was also explored by inhibiting p53 expression in HK2 cells. Cisplatin-induced mtROS was significantly inhibited in cells transfected with p53 siRNA (Fig. 7A and 7B). Through double staining with the mitochondria-specific marker MitoTracker Red and the ROS probe DCFDA, the mitochondrial and ROS signals were simultaneously visualized (Fig. 7C). Consistent with the result obtained by using MitoSOX, p53 siRNA could dramatically deplete the DCF fluorescence induced by cisplatin (Fig. 7C and 7D). Also, inhibition of p53 by siRNA resulted in cell survival as revealed by CCK-8 (Fig. 7E), TUNEL assay (Fig. 7F) and flow cytometry (Fig. 7G). Taken together, the results indicated that p53 mediated cisplatin-induced mtROS and cytotoxicity in HK2 cells by regulating the expressions of MnSOD and p53.
p53 inhibitor restored MnSOD expression and inhibited p66shc expression during cisplatin nephrotoxicity

We further sought in vivo support for the the effect of p53 on mtROS and cell damage by regulating MnSOD and p66shc by studying mice that had been treated with cisplatin for 3 days. As shown in Fig. 8A and B, the p53 inhibitor pifithrin-α markedly inhibited cisplatin-induced p53 expression. In addition, Pifithrin-α reduced p66shc expression and restored MnSOD expression at both the transcriptional and posttranslational levels following cisplatin administration. These studies indicate that the p53-dependent regulation of MnSOD and p66shc genes in vivo is similar to that observed in cell cultures. Similarly, cisplatin induced the phosphorylation, acetylation and ubiquitylation of p53 (Fig. 8C), suggesting that the posttranslational modification of p53 might play critical roles in cisplatin nephrotoxicity.

p53 inhibitor ameliorates cisplatin-induced renal failure

At day 3, mice treated with cisplatin developed severe renal failure as shown by high levels of BUN (130.1 mg/dl) and serum creatinine (0.44 mg/dl) and Pifithrin-α significantly
ameliorated cisplatin-induced acute kidney injury (AKI) (Fig. 9A and 9B). The improvement in renal function was also reflected in kidney histology. Pifithrin-α facilitated significant recovery from the severe tubular injury induced by cisplatin (Fig. 9C and 9D).

**p53 inhibitor blocked cisplatin-induced oxidative stress and apoptosis in the kidney**

Finally, we established the role of p53 on ROS and apoptosis in cisplatin-treated renal tubular epithelial cells in vivo. As shown in Fig. 10A and 10B, cisplatin increased the number of apoptotic cells, and Pifithrin-α significantly decreased the cisplatin-induced apoptosis. Furthermore, malondialdehyde (MDA) levels, the indicator used to assess the pro-oxidative status in kidneys, were increased in cisplatin-treated mice, but were improved in the Pifithrin-α-treatment group (Fig. 10C). Thus, these tests supported that p53 mediated cisplatin-induced oxidative stress and apoptosis in the kidney.
Discussion

In the present study we demonstrate that in HK2 cells, cisplatin exposure decreased the MnSOD and increased the expression of p53 and p66shc, and cisplatin-induced mtROS generation and cell injury were abolished by using MnSOD mimic and p66shc siRNA, respectively. In addition, down regulation of p53 reversed the effects of cisplatin on mtROS and cell injury by restoring MnSOD and inhibiting p66shc. Furthermore, in vivo studies with mice also revealed that administration of p53 inhibitor reduced cisplatin-induced oxidative stress and apoptosis in the kidney by regulating MnSOD and p66shc.

Acute kidney injury (AKI) can develop in a variety of clinical situations such as ischemia, sepsis, and administration of nephrotoxic agents [22]. The most representative group of chemicals that cause AKI includes cancer chemotherapeutics such as cisplatin and doxorubicin. Cisplatin is used for the treatment of testicular and ovarian cancers, but leads to nephrotoxicity [23]. Cisplatin treatment damages the proximal tubules both in vivo and in vitro [24]. Multiple factors and pathways are involved in acute kidney injury resulting
from cisplatin treatment. Apoptosis is a major cause of tubular cell loss in cisplatin-induced AKI [25]. Cisplatin can induce apoptosis via both death receptor pathway and mitochondrial pathway [26]. Consistent with previous studies [27], our results showed that cisplatin induced apoptosis in HK2 cells and the kidney, as shown by a significant induction in the number of TUNEL-positive cells. These findings suggest that the inhibition of apoptosis may be a powerful therapeutic strategy for the prevention and treatment of cisplatin nephrotoxicity.

ROS, particularly the superoxide radical, is important in cisplatin-induced nephrotoxicity [28]. A variety of cellular enzyme systems are potential sources of ROS, including NADPH oxidase, xanthine oxidase, uncoupled endothelial nitric oxide (NO) synthase (eNOS), arachidonic acid metabolizing enzymes including cytochrome P-450 enzymes, lipoxygenase and cyclooxygenase, and the mitochondrial respiratory chain [29]. The mitochondrial
showed that cisplatin treatment increased mtROS production via the disrupted respiratory chain in porcine proximal tubular cells [31]. Consistently, our results also found that cisplatin triggers the opening of the mitochondrial permeability transition pore and induces apoptosis and/or necrosis [32].

Oxidative stress is imbalance between ROS generation and antioxidant system that scavenge ROS levels. The mitochondrial antioxidant enzymes included MnSOD, peroxiredoxin III (Prx3), peroxiredoxin V (Prx5), mitochondrial thioredoxin (Trx2), and mitochondrial thioredoxin reductase (TrxR2). MnSOD is the primary mitochondrial antioxidant enzyme and is essential for maintaining normal cell development and function. Several studies have shown that overexpression of MnSOD was beneficial in various animal models and cultured cells [33]. More importantly, in cisplatin-induced renal cell injury, overexpression of MnSOD provides protection against cisplatin-mediated cytotoxicity in cultured renal epithelial cells [34]. Our data support these studies that MnSOD decreased after cisplatin treatment both in vivo and in vitro, and MnTBAP, a MnSOD mimic, blocked cisplatin-induced the generation of superoxide and cell injury, suggesting poor antioxidant response was induced by cisplatin and MnSOD acts as a survival strategy against cisplatin-induced tubular cell injury.

**Fig. 8.** Effect of Pifithrin-α on MnSOD, p66shc, p53 expressions and p53 post-translation modification in cisplatin-induced acute kidney injury. Kidney samples were collected 72 h after cisplatin treatment. (A) MnSOD, p66shc and p53 mRNA expression by real-time RT-PCR analysis. (B) MnSOD, p66shc and p53 protein expression by immunoblotting analysis. Upper: representative immunoblots. Lower: densitometric analysis. (C) p53 phosphorylation, p53 acetylation and Mdm2 levels were detected by immunoblotting. Upper: representative immunoblots. Lower: densitometric analysis. Values represent means ± SEM, n = 6. *P < 0.01 versus control group, #P < 0.01 versus cisplatin-treated group by ANOVA. Cntl, control group; Pif, Pifithrin-α group; Cisplatin, cisplatin group; Cisplatin+Pif, cisplatin+ Pifithrin-α group.
P66shc-induced mitochondrial ROS have been shown to be involved in a wide variety of pathologies. p66shc is activated by phosphorylation at its Serine36 residue, which promotes its translocation to the mitochondrial intermembrane space and induces production of hydrogen peroxide by utilizing the mitochondrial electron transfer chain [35]. It has been reported that overexpression of p66shc exacerbates while its knockdown or mutation of the Serine36 site to alanine ameliorates cisplatin-induced nephrotoxicity in renal proximal tubule cells [36]. Our current study confirmed that cisplatin induced the expression of p66Shc and suppression of endogenous p66Shc by siRNA blocked cisplatin-induced mtROS
Fig. 10. Effects of Pifithrin-α on cell apoptosis and oxidative stress in cisplatin-induced acute kidney injury. (A) Representative images of the TUNEL assay show cisplatin-induced renal tubular cell apoptosis, which is attenuated in Cisplatin+Pif group (Original magnification, ×400). (B) Quantitation of TUNEL-positive nuclei in the tissues from each condition. Treatment with Pifithrin-α significantly reduced the number of TUNEL-positive nuclei. (C) Malondialdehyde (MDA) levels in kidneys. *P < 0.01 versus control group, #P < 0.01 versus cisplatin-treated group by ANOVA. Cntl control group; Pif, Pifithrin-α group; Cisplatin, cisplatin group; Cisplatin+Pif, cisplatin+Pifithrin-α group.

Fig. 11. A schematic model of the proposed links between p53, p66shc, MnSOD and mtROS in renal tubular cells after cisplatin treatment. The activation of p53 induced by cisplatin enhanced the generation of mtROS by up regulating p66shc and down regulating MnSOD, finally contributing to renal tubular cells injury. p53, p66shc, MnSOD and mtROS are potential therapeutic targets for cisplatin nephrotoxicity.

production and cell injury. These data indicated another possibility that cisplatin-induced mtROS production and cell damage in HK-2 cells was related to the enhancement of p66shc.
Cisplatin plays an important role in up regulating p53 expression in cancer cells [37]. Also, p53 has become an important pathogenic mechanism in cisplatin-induced nephrotoxicity [38]. p53 regulates cell death and survival through both transcription-dependent and -independent mechanisms [39]. p53 may induce apoptosis by directly activating proapoptotic Bcl-2 family proteins, leading to mitochondrial injury and the release of apoptogenic factors [40]. Furthermore, p53 has been demonstrated to be upstream of MnSOD and p66Shc. P53-null mice displayed attenuated p66shcA expression and diminished ROS generation [41]. Supporting the notion, we also found that the effects of cisplatin on the expression of MnSOD and p66shc were reversed by p53 inhibition both in vivo and in vitro. As recently reviewed, p53 activity is not only determined by its expression levels, but also by a number of post-translational modifications such as phosphorylation, acetylation, and ubiquitination [42]. These modifications can dictate the p53 response to diverse cellular signals and help determine its physiological activities. Our current study confirmed that cisplatin induced p53 phosphorylation and acetylation, and p53 activation mediated mtROS production by regulating p66shc and MnSOD. Interestingly, we found that cisplatin also activate p53 ubiquitination for degradation. However, up regulation of p53 ubiquitination by Mdm2 cannot abolish the effect of cisplatin on p53 activation. Therefore, further studies are necessary to elucidate the crosstalk among different modifications.

In conclusion, our study provides evidence that in cisplatin nephrotoxicity, p53 signaling helps inhibit MnSOD and activate p66shc, which mediate mtROS generation and finally lead to tubular cells damage. Inhibiting p53 ameliorates acute kidney injury by restoring MnSOD and inhibiting p66shc, thereby blocking mtROS production and cell injury (Fig. 11). The current studies define a new mechanism by which p53 signaling affects mtROS in acute kidney injury.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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