Role of p53 in cisplatin-induced tubular cell apoptosis: dependence on p53 transcriptional activity

Man Jiang, Xiaolan Yi, Stephen Hsu, Cong-Yi Wang, and Zheng Dong.

1Department of Cellular Biology and Anatomy, 2Department of Oral Biology and Maxillofacial Pathology, and 3Center for Genomic Medicine, Medical College of Georgia, Augusta, Georgia 30912; and 4Medical Research Service, Department of Veterans Affairs Medical Center, Augusta, Georgia 30904

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Tubular damage by cisplatin leads to acute renal failure, which limits its use in cancer therapy. In tubular cells, a primary target for cisplatin is presumably the genomic DNA. However, the pathway relaying the signals of DNA damage to tubular cell death is unclear. In response to DNA damage, the tumor suppressor gene p53 is induced and is implicated in subsequent DNA repair and cell death by apoptosis. The current study was designed to examine the role of p53 in cisplatin-induced apoptosis in cultured rat kidney proximal tubular cells. Cisplatin at 20 μM induced apoptosis in ~70% of cells, which was partially suppressed by carbobenzoxy-Val-Ala-Asp-fluoromethyl ketone (VAD), a general caspase inhibitor. Of interest, cisplatin-induced apoptosis was also suppressed by pifithrin-α, a pharmacological inhibitor of p53. Cisplatin-induced caspase activation was completely inhibited by VAD, but only partially by pifithrin-α. Early during cisplatin treatment, p53 was phosphorylated and upregulated. The p53 activation was blocked by pifithrin-α, but not by VAD. Bcl-2 expression ablated cisplatin-induced apoptosis without blocking p53 phosphorylation or induction. The results suggest that p53 activation might be an early signal for apoptosis during cisplatin treatment. To further determine the role of p53, tubular cells were stably transfected with a dominant-negative mutant of p53 with diminished transcriptional activity. Expression of the mutant attenuated cisplatin-induced apoptosis and caspase activation. In conclusion, the results support an important role for p53 in cisplatin nephrotoxicity.

Our study was designed to further investigate p53 regulation by cisplatin and the role played by p53 in cisplatin-induced tubular cell apoptosis. Using pharmacological and molecular approaches, we show that p53 activation is an early signal for tubular cell injury following cisplatin exposure. Importantly, the results suggest that p53 may mediate tubular cell apoptosis through its regulation of gene transcription.

MATERIALS AND METHODS

Materials. A rat kidney proximal tubular cell line was obtained from Dr. U. Hopfer (Case Western Reserve University, Cleveland, OH) and maintained for experiments as described previously (50). Carbobenzoxy-Val-Ala-Asp-fluoromethyl ketone (VAD), carbobenzoxy-Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD.AFC), and 7-amino-4-trifluoromethyl coumarin (AFC) were purchased from Enzyme Systems Products (Dublin, CA). The rabbit polyclonal antibody specific to the active form of caspase-3 was a generous gift from Dr. A. Srinivasan at Idun Pharmaceuticals (La Jolla, CA). Rabbit polyclonal anti-p53 and anti-phospho-p53 (Ser15) antibodies were purchased from Cell Signaling Technology (Beverly, MA) and BD Pharmingen (San Diego, CA). Secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, PA). Other reagents including cisplatin were from Sigma (St. Louis, MO).

Stable transfection of renal tubular cells with dominant-negative p53. The dominant-negative mutant of p53 with a point mutation (V143A) was a generous gift from Dr. Chi-Hui Tang (Department of Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center).
Center and Health Science Center, Houston, TX). The mutant was originally from Biomyx Technology (San Diego, CA). The mutant sequence was subcloned into pcDNA3.1 that contained a hygromycin resistance cassette using HindIII and XbaI. Transfection was conducted as previously (52). Briefly, tubular cells were plated at 1.5 × 10⁶/60-mm dish to reach 60–70% confluence after overnight growth and transfected with pcDNA3.1-p53-DN using Lipofectam 2000 (Invitrogen). After transfection, the cells were maintained in culture medium containing 400 μg/ml hygromycin for 7–10 days. Hygromycin-resistant cell colonies were identified and transfected to separate dishes by trypsinization in cloning cylinders. These clones were expanded and tested for the expression of dominant-negative p53 by immunoblotting. Positive clones were subjected to a second round of cloning to ensure the selection of pure colonies.

**Cisplatin incubation.** Cells were plated at a density of 1–1.4 × 10⁶ per 35-mm dish. After 24 h of growth, the dish reached 80–90% confluence. For experiments, the cells were washed with phosphate-buffered saline and incubated with 20 μM cisplatin in fresh culture medium. Cisplatin was freshly prepared for each experiment. By the end of incubation, cells were monitored morphologically or lysed with indicated buffers to collect cell lysate for biochemical analyses. For cell lysis, both floating and adherent cells were collected.

**Morphological examination of apoptosis.** Apoptosis was monitored morphologically, as described in our recent publications (8, 47, 48). Typical apoptotic morphology examined included cellular shrinkage, nuclear condensation and fragmentation, and formation of apoptotic bodies. Briefly, cells were exposed to 10 μg/ml of Hoechst 33342 for 2–5 min to stain the nuclei. Cellular and nuclear morphology were then monitored by phase-contrast and fluorescence microscopy. Four fields with ~200 cells per field were checked in each 35-mm dish to estimate the percentage of cells with typical apoptotic morphology.

**Enzymatic assay of caspase activity.** We used DEVD.AFC, a fluorogenic peptide substrate of caspases, to measure the enzymatic activity of caspases in cell lysates (8, 47, 48). Briefly, cells were extracted with 1% Triton X-100. The Triton lysates of 25 μg protein were added to enzymatic reactions containing 50 μM DEVD.AFC. After 60 min of reaction at 37°C, fluorescence at excitation 360 nm/emission 530 nm was monitored by a GENios plate-reader (Tecan). For each measurement, a standard curve was constructed using free AFC. Based on the standard curve, the fluorescence reading from each enzymatic reaction was translated into the nanomolar amount of liberated AFC to indicate caspase activity.

**Immunofluorescence.** Indirect immunofluorescence was conducted as described in our previous work (8, 9, 47, 48). Cells were grown on the collagen-coated glass coverslip. For caspase-3, cells were fixed with 4% paraformaldehyde. For p53 and phospho-p53 (serine-15), cells were fixed with a modified Zamboni’s fixative containing picric acid and 4% paraformaldehyde. After fixation, the cells were incubated with a blocking buffer containing 2% normal goat serum. The cells were then incubated with primary antibodies, followed by exposure to Cy3-labeled goat-antirabbit secondary antibody. Signals were examined by fluorescence microscopy using a Cy3 channel.

**Immunoblot analysis.** Whole cell lysates was collected with 2% SDS buffer. Protein concentration was determined using the bicinchoninic acid (BCA) reagent from Pierce Chemical (Rockford, IL). The same amounts (usually 25 μg) of protein were loaded for each lane for electrophoresis under reducing condition. Subsequently, resolved proteins were electroblotted onto PVDF membranes. The blots were blocked in 1% BSA and 2% fat-free milk and then exposed to the primary antibodies overnight at 4°C. Finally, the blots were incubated with the horseradish peroxidase-conjugated secondary antibody, and antigens on the blots were revealed using an enhanced chemiluminescence (ECL) kit from Pierce.

**Statistics.** Data were expressed as means ± SD (n ≥ 4). Statistical differences between two groups were determined using the Student’s t-test with Microsoft EXCEL 2002. P < 0.05 was considered to reflect significant differences.

**RESULTS**

Cisplatin-induced apoptosis is partially suppressed by VAD and pifithrin-α. Depending on its concentration, cisplatin induces necrotic as well as apoptotic forms of cell death in renal cells (3, 7, 13, 19, 23, 31, 33, 35, 36, 40, 41, 44). Consistently, in our experiments, 20 μM cisplatin induced significant amounts of apoptosis, whereas a higher concentration (50–100 μM) led to both necrosis and apoptosis (not shown). As shown in Fig. 1A, after 20 μM cisplatin treatment, the cells assumed a shrunken configuration with noticeable apoptotic bodies. Cell nuclei were also condensed and fragmented, as shown by Hoechst staining. Necrosis was minimal, as shown by the integrity of plasma membrane that excluded propidium iodide of 668 Da (not shown). Apoptotic cells were quantified based on their characteristic morphology. Results of six experiments are summarized in Fig. 1B. Cisplatin incubation for 24 h induced 68% apoptosis, which, not surprisingly, was suppressed to 24.5% by 100 μM VAD, a general inhibitor of caspases. Of interest, cisplatin-induced apoptosis was also suppressed by pifithrin-α (PF), a pharmacological inhibitor of p53 (15). The inhibition by PF was dose dependent in the range of 1–20 μM (not shown). In the presence of 20 μM PF, the apoptosis rate was reduced to 27.5%. The effects of PF at higher concentrations were not examined due to the limited solubility of the chemical (not shown).

**Caspase activation by cisplatin is completely inhibited by VAD and partially inhibited by PF.** Our experiments showed that 100 μM VAD did not completely block cisplatin-induced apoptosis in cultured tubular cells (Fig. 1). We wondered whether VAD at the given concentrations was able to completely inhibit caspase activation. The enzymatic activity of caspases was measured using the fluorogenic peptide substrate DEVD.AFC. As shown in Fig. 2A, cisplatin induced a drastic increase in caspase activity. In the presence of 100 μM VAD, caspase activation was completely abolished, to a level lower than the control group without cisplatin exposure. PF also inhibited caspase activation during cisplatin treatment. However, the inhibition by PF was ~50% and not complete. We further examined the formation of the active fragments of caspase-3 under these conditions by immunofluorescence. Cell morphology of the same fields was recorded to determine the (in) correlation between apoptosis and caspase activation. The results are shown in Fig. 2B. In control, apoptosis was minimal and very few cells had positive staining for active caspase-3. Cisplatin incubation led to significant apoptosis and positive staining of active caspase-3. Of note, virtually every cell with apoptotic morphology was positive for active caspase-3. In the presence of VAD, apoptosis was reduced, and so was the number of active caspase-3-positive cells. However, the remaining apoptotic cells still had positive staining of the active caspase-3 fragment, albeit at a lower intensity. As will be discussed, in these cells caspase-3 might be processed into active fragments by caspase-independent mechanisms; the fragments were saturated by VAD and did not show enzymatic activities (Fig. 2A). The fact that VAD completely blocked caspase activation (Fig. 2A) but could not completely inhibit
apoptosis (Fig. 1) suggests the presence of caspase-independent apoptosis in our experimental model. For PF, the inhibition on apoptosis as well as active caspase-3 staining was partial (Fig. 2B).

Fig. 1. Inhibition of cisplatin-induced apoptosis by carbobenzyoxy-Val-Ala-Asp-fluoromethyl ketone (VAD) and pifithrin-α (PF). Cultured renal tubular cells were incubated with 20 μM cisplatin for 24 h in the absence or presence of 100 μM VAD or 20 μM PF. Control group was cultured for the same period of time without cisplatin exposure. The cells were then fixed with 4% paraformaldehyde and stained with Hoechst 33342. A: representative images of cell morphology and nuclear staining of the same fields of cells. B: percentage of cells that showed typical apoptotic morphology. Data are expressed as means ± SD (n = 6). *Statistically significantly different from the control. #Statistically significantly different from the cisplatin-only group. The results show that tubular cell apoptosis during cisplatin incubation was partially suppressed by VAD and PF.

Fig. 2. Inhibition of caspase activation during cisplatin treatment by VAD and PF. Cells were incubated with 20 μM cisplatin for 24 h in the absence or presence of 100 μM VAD or 20 μM PF. Control group was cultured for the same period of time without cisplatin exposure. A: caspase activity measured by enzymatic assays using DEVD.AFC as the substrate. Data are expressed as means ± SD (n = 4). *Statistically significantly different from the control. #Statistically significantly different from the cisplatin-only group. B: cell morphology and immunofluorescence of active caspase-3. Cells were fixed and processed for immunofluorescence using an antibody that specifically reacted with the active fragments of caspase 3. The results show that caspase activation during cisplatin treatment was completely diminished by VAD and partially by PF.

p53 IS phosphorylated and induced early during cisplatin treatment. To further examine the role of p53 in cisplatin nephrotoxicity, we analyzed p53 activation by immunoblotting. Our first series of experiments showed that p53 was
induced early following cisplatin incubation. As shown in Fig. 3A, p53 induction became detectable after 2-h cisplatin exposure. By 4 h, consistent p53 induction was demonstrated, 1.7-fold over control (Fig. 3B). At the end of 24-h cisplatin incubation, p53 was 3.5-fold over control (Fig. 3B). We subsequently determined p53 phosphorylation, which is an important regulatory mechanism for p53. As shown in Fig. 3A, cisplatin exposure led to evident p53 phosphorylation at the site of serine-15. By 4 h, phospho-p53 (p-p53) was about fourfold over control and increased further as cisplatin incubation was prolonged (Fig. 3C). Phosphorylation of p53 at other sites was also detected (not shown). We further monitored time dependence of apoptosis and caspase activation induced by cisplatin. As shown in Fig. 3, D and E, noticeable apoptosis and caspase activation were not detected until 8-h cisplatin incubation. Thus, in renal tubular cells, p53 was activated early following cisplatin incubation, before apoptosis.

p53 Activation by cisplatin is blocked by PF but not by VAD. The time course of p53 activation by cisplatin (Fig. 3) suggested that p53 was not activated as a result of apoptosis. To further examine this, we analyzed the effects of VAD and PF on p53 activation. The results are shown in Fig. 4. Consistent with previous observations, p53 was induced and phosphorylated on cisplatin exposure (Fig. 4A, lane 2). As expected, both p53 phosphorylation and induction were attenuated by PF (lane 3). On the contrary, VAD, while suppressing apoptosis and caspase activation (Figs. 1 and 2), did not block p53 activation (lane 4). The immunoblotting results were confirmed by immunofluorescence analysis. As shown in Fig. 4B, faint p53 staining was detected in control cells and was mainly in their nuclei. After cisplatin exposure, many cells showed intense p53 staining, which was also in the nucleus. PF suppressed the p53 staining to some extents, whereas VAD was largely ineffective. The immunofluorescence of p-p53 was also examined. In control, very few cells had p-p53 staining. The number of cells with p-p53 was significantly increased during cisplatin incubation; such an increase was ameliorated by PF but not by VAD.

Bcl-2 attenuates cisplatin-induced apoptosis without inhibiting p53 activation. Bcl-2 is a prototypical antiapoptotic protein. In renal tubular cells, we showed that transfection and pharmacological induction of Bcl-2 protect against apoptosis induced by a variety of insults (38, 47). We therefore tested cisplatin injury in tubular cells that were stably transfected with Bcl-2. As shown in Fig. 5A, whereas 69% apoptosis was induced by cisplatin in wild-type (WT) cells, less than 5% apoptosis was detected in Bcl-2 cells. Of note, in Bcl-2 cell, the spontaneous apoptosis under control conditions was also lower. We then analyzed whether Bcl-2 blocked apoptosis by suppressing p53 activation during cisplatin treatment. The results are shown in Fig. 5B. Clearly, p53 was phosphorylated and induced in Bcl-2 cells following cisplatin exposure. Den- sitometric analyses of the blots indicate that p53 activation in Bcl-2 cells was not lower but slightly higher than that in WT cells (not shown). For example, 8 h of cisplatin induced a threefold increase in p53 in Bcl-2 cells and about twofold in WT cells. The results suggest that Bcl-2 ameliorated cisplatin-induced apoptosis at a level downstream of p53.
cisplatin treatment were attenuated by PF but not by VAD.

METHODS. The results show that p53 phosphorylation and induction during cisplatin-induced apoptosis in renal tubular cells. To further determine the role of p53, we decided to target p53 more specifically by expressing dominant-negative (DN) mutants of this gene. After analyzing the functional domains of p53, we chose to use a DN-p53 with a point mutation in the DNA binding region. This mutation has been shown to prevent DNA binding and, in turn, block gene-transcriptional activity of p53 (43, 49). The DN-p53 was transfected into renal tubular cells to select stably transfected clones. The selected clones were analyzed by immunoblotting to verify the expression of the p53 mutant. As shown in Fig. 6A, DN-p53 was expressed in clones 4, 5, 6, 7, 8, and 10, but not in clone 9. We then conducted an initial screening experiment to test cisplatin-induced apoptosis in these cells. All of the DN-p53 transfected clones showed less apoptosis than WT cells. Clones 4, 5, and 10 appeared particularly resistant to cisplatin injury (data not shown). Their resistance was not due to the lack of p53 induction during cisplatin incubation, as shown in Fig. 6B.

DN-p53 abolishes cisplatin-induced apoptosis and caspase activation. Based on the initial screening, we further compared clones 4, 5, and 10 with WT tubular cells for apoptosis following cisplatin treatment. The results are summarized in Fig. 7A. The basal level of apoptosis in WT cells was ~3%, and after 24-h cisplatin incubation, apoptosis was increased to 68%. In DN-p53 clones, basal levels of apoptosis were all below 1% (not shown). Importantly, cisplatin-induced apoptosis in these three clones was between 7 and 12%, significantly lower than that of WT cells (#, significantly lower than WT). Cell morphology of a typical experiment is shown in Fig. 7B. Both cellular and nuclear features of apoptosis were suppressed in DN-p53 clones. We further analyzed caspase activity in these cells. The results are shown in Fig. 7C. Clearly, caspase activation by cisplatin in DN-p53-transfected cells was significantly lower than that of WT cells (#, significantly lower than WT). Of note, transfection of DN-p53 cells did not completely block apoptosis and caspase activation during cisplatin incubation (*, significantly higher than the basal level of control cells), suggesting the presence of p53-independent mechanisms of apoptosis.
DISCUSSION

After cisplatin exposure, p53 is induced and accumulates in the cell nucleus in vivo in the kidneys (24). A recent study using the pharmacological inhibitor PF further suggested a role for p53 in cisplatin-induced tubular cell damage (7). Our results extended these observations. First, we demonstrated early p53 phosphorylation and induction during cisplatin treatment. Second, while the general caspase inhibitor VAD and the antiapoptotic gene Bcl-2 ameliorated cisplatin-induced apoptosis, they did not inhibit p53 activation, suggesting that p53 activation was not a consequence of cell death. On the other hand, PF blocked p53 activation and attenuated apoptosis, suggesting that p53 might be an early signal for cisplatin-induced cell death. Finally, DN-p53 abolished apoptosis during cisplatin incubation, further supporting a role for p53 in nephrotoxicity of this chemotherapy drug. Importantly, the mutation in the DN-p53 was at the DNA binding site and was shown to diminish its transcriptional activity. Thus the inhibitory effects of the mutant suggest that p53 may mediate cisplatin injury by regulating the transcription of apoptotic genes.

Tubular cell death induced by cisplatin can take place in the form of necrosis or apoptosis (14, 19, 35, 37, 40). One factor that determines the morphological outcome is the severity of the injury. It has been shown that cisplatin at high micromolar concentrations (usually >100 μM) triggers necrosis, whereas at lower concentrations it leads to apoptosis (19). Whether cells undergo necrosis or apoptosis is also dependent on the condition or context of the cells (17). For example, apoptosis is an active process that requires energy to complete the execution program. Thus, if the cells experience severe ATP depletion during injury, then apoptosis cannot progress and necrosis ensues. These considerations are relevant to the in vivo situation of renal tubules, which, despite the enormous ability of aerobic respiration, possess very limited capacity of anaerobic glycolysis (18). Under such conditions, renal tubules programmed to apoptosis may end up with necrosis due to toxic interruption of aerobic respiration. This is probably one of the explanations for the impressive numbers of necrotic cells that are usually identified in renal tubules in the kidneys following cisplatin damage. In vitro, cultured renal tubules and certainly the tubular cell lines have acquired the phenotypic alterations to facilitate glycolytic ATP generation for apoptosis. Consistently, in our experiments, 20 μM cisplatin induced apoptosis and not necrosis, as shown by the integrity of plasma membrane that excluded propidium iodide (data not shown).

The pathway leading to tubular cell apoptosis by cisplatin is unclear. Caspase activation initiated by death receptors including Fas and TNF-2 receptor has been demonstrated in in vitro and in vivo models of cisplatin nephrotoxicity (36, 37, 44). On the other hand, the mitochondrial pathway of apoptosis involving Bax translocation and cytochrome c release also appears to play an important role (16, 33). Recent work further suggested the involvement of endoplasmic reticulum stress and the associated activation of caspase-12 (2). An important development in this area is the recognition of cells that assume an apoptotic morphology in a caspase-independent manner (7, 31). In our experiments, cisplatin induced a significant activation of caspase-3. In this area is the recognition of cells that assume an apoptotic morphology in a caspase-independent manner (7, 31). In our experiments, cisplatin induced a significant activation of caspases. By immunofluorescence, we showed that virtually every cell with apoptotic morphology had active caspase-3. In the presence of VAD, caspase activation was completely blocked, and yet, inhibition of apoptosis was not complete (Figs. 1 and 2). The results support previous observations by other investigators that cisplatin may induce caspase-independent apoptosis (7, 31). With VAD, the remaining apoptotic cells still had positive staining for the active caspase-3 fragment, albeit at a lower intensity (Fig. 2B). Apparently, in these cells, caspase-3 was proteolytically processed by caspase-independent mechanisms. The caspase-3 fragment was satu-
rated by VAD and did not exhibit enzymatic activity. The development of apoptotic morphology in these cells was thus caspase independent.

The conclusion that p53 is an early signal for, rather than a consequence of, cisplatin-induced apoptosis is based on the following observations. First, p53 activation occurred early during cisplatin incubation. We detected p53 phosphorylation and induction as early as 2 h of cisplatin exposure (Fig. 3). By 4 h, consistent p53 activation was detected. In these cells, apoptosis remained minimal, comparable to control group without cisplatin exposure. Second, VAD suppressed apoptosis from ∼70 to 25%, without inhibiting p53 activation (Fig. 4). Importantly, in cells expressing Bcl-2, cisplatin-induced apoptosis was completely diminished. However, Bcl-2 did not attenuate p53 phosphorylation or induction (Fig. 5). These results suggest that VAD and Bcl-2 block apoptosis at levels downstream of p53. Third, we showed that pharmacological inhibition of p53 with PF abrogated apoptosis during cisplatin incubation (Fig. 1). This observation is consistent with recent work by Cummings and Schnellmann (7). Importantly, we further showed that blockade of p53 with a dominant-negative mutant suppressed apoptosis as well as caspase activation (Figs. 6 and 7). These results, together with previous observations, provide strong evidence for a role of p53 in cisplatin nephrotoxicity.

The regulation of p53 is very complex (10, 46). In renal tubular cells, despite the recognition of p53 induction by cisplatin (7, 24), the underlying mechanism has yet to be investigated. Posttranscriptional mechanisms including phosphorylation and acetylation might play an important role. For example, phosphorylation of p53 may prevent its ubiquitination by MDM2 and as a result prevent p53 degradation via proteasome. Our experiments demonstrated a drastic increase in p53 phosphorylation at serine-15. Importantly, the time course of serine-15 phosphorylation coincided with that of p53 induction. We also showed p53 phosphorylation at other sites (data not shown), suggesting a complex posttranscriptional regulation of p53 under these experimental conditions. Currently, it is unclear which phosphorylation site(s) is critical for p53 induction. Moreover, the protein kinases responsible for p53 phosphorylation remain to be identified. Nevertheless, our results suggest a role for posttranscriptional regulation of p53 in its induction during cisplatin nephrotoxicity.

The downstream molecular targets of p53 that trigger apoptosis have been a hot area of research pursuit (10, 21, 46). Thus far, two types of p53 regulation have been identified. First, p53 may induce the transcription and expression of apoptotic genes. In this direction, p53 induces the expression of death receptors including Fas and DR5 during cell incubation with chemotherapy drugs including cisplatin (27, 51). High levels of death receptors presumably sensitize the cells to apoptosis following ligand binding. If overexpressed, the death receptors may initiate cell death by themselves, even in the absence of ligands. p53 Also upregulates the expression of proapoptotic genes in the mitochondrial-mediated pathway of apoptosis. Particularly, the proapoptotic members of Bcl-2 family protein including Bax, Puma, Noxa, and Bid appear to be regulated by p53 and may have important roles in p53-initiated apoptosis (26, 28, 32, 39, 53). Using knockout models, recent studies provided compelling evidence that p53-mediated apoptosis may depend on the expression of Puma and Noxa (45). Alternatively, p53 may directly activate the apoptotic cascade in a gene transcription-independent manner. It has been reported that p53 translocates to mitochondria to induce membrane damage in the organelles (22, 25). Interestingly, recent work further showed that p53 directly activated Bax in the cell cytosol to permeabilize mitochondria and release apoptogenic factors, resulting in typical apoptosis (4). Of note, in this study, the apoptosis-activating action of p53 was independent of its nuclear location or transcriptional activity. In our experiments, the dominant-negative mutant of p53 had a point mutation in the DNA binding domain that diminished its transcriptional activity (43, 49). The expression of this mutant blocks WT p53-mediated gene transcription, as shown by previous studies (43, 49). Thus the observed inhibitory effects of this mutant suggest that p53 may regulate cisplatin-induced tubular apoptosis by transcription of apoptotic genes.

In conclusion, this study has shown p53 phosphorylation and induction during cisplatin treatment of renal tubular cells. Inhibitory experiments using pharmacological and molecular approaches have provided compelling evidence for a role of p53 in cisplatin-induced tubular cell apoptosis. p53 may regulate cisplatin nephrotoxicity through gene transcription.

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REFERENCES


25. Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, Miyashita T, and Reed JC. Tumor suppressor p53 is a direct transcrip-


38. Tang CH and Grimm EA. Depletion of endogenous nitric oxide enhances cisplatin-induced apoptosis in a p53-dependent manner in melano-


40. Villunger A, Michalak EM, Coutlas L, Mullauer F, Bock G, Assuer-


42. Wang J, Wei Q, Wang CY, Hill WD, Hess DC, and Dong Z. Minocyc-


