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Perspectives in Pharmacology

Regulation and Pathological Role of p53 in Cisplatin Nephrotoxicity

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ABSTRACT

Cisplatin is one of the most potent chemotherapy drugs widely used for cancer treatment. However, its use is limited by side effects in normal tissues, particularly the kidneys. Recent studies, using both in vitro and in vivo experimental models, have suggested a critical role for p53 in cisplatin nephrotoxicity. The signaling pathways upstream and downstream of p53 are being investigated and related to renal cell injury and death. Along with the mechanistic studies, renoprotective approaches targeting p53 have been suggested. Further research may integrate p53 signaling with other nephrotoxic signaling pathways, providing a comprehensive understanding of cisplatin nephrotoxicity and leading to the development of effective renoprotective strategies during cancer therapy.

Cisplatin is one of the most effective chemotherapeutic agents widely used for the treatment of malignant tumors in testis, ovary, cervix, lung, head and neck, bladder, and many other organs and tissues (Siddik, 2003; Wang and Lippard, 2005; Cepeda et al., 2007). However, the use and efficacy of cisplatin is limited by its side effects, including nephrotoxicity, neurotoxicity, ototoxicity, hair loss, nausea, and vomiting (Taguchi et al., 2005; Wang and Lippard, 2005; Cepeda et al., 2007). As a principal site for drug filtration, concentration, and excretion, renal tissues and cells are exposed to toxic concentrations of cisplatin during cancer therapy (Arany and Safirstein, 2003). As a result, nephrotoxicity is the major dose-limiting factor during cisplatin treatment (Meyer and Madias, 1994; Arany and Safirstein, 2003; Hanigan and Devarajan, 2003; Taguchi et al., 2005; Pabla and Dong, 2008). Clinical data have shown that approximately one-third of patients experience kidney injury in days after cisplatin treatment, with reduced glomerular filtration rate, increased blood urea nitrogen and serum creatinine, and imbalanced electrolyte (Meyer and Madias, 1994; Hanigan and Devarajan, 2003). Renal tubular cell injury and death are the key pathological occurrences in cisplatin nephrotoxicity (Arany and Safirstein, 2003; Pabla and Dong, 2008). Depending on cisplatin concentration and/or cellular status, tubular cell death in the forms of both apoptosis and necrosis has been recognized (Lieberthal et al., 1996; Pabla and Dong, 2008), although the mechanism underlying cisplatin-induced tubular cell death is not fully understood.

Cisplatin is a well recognized DNA-damaging agent, and it is generally accepted that genomic DNA is the primary target of the drug (Siddik, 2003; Wang and Lippard, 2005; Cepeda et al., 2007). Once inside the cell, cisplatin undergoes aquation reactions, converting into a positively charged and highly reactive molecule that interacts with DNA to form the cisplatin-DNA adducts inducing intrastrand and interstrand cross-linking. The cross-linking distorts and abnormally unwinds DNA duplex, interfering with DNA replication and/or transcription, resulting in DNA damage that triggers cell-cycle arrest and cell death (Jamieson and Lippard, 1999; Wang and Lippard, 2005). In tumors and cancer cells, cisplatin-induced DNA damage has been recognized as the major

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ABBREVIATIONS: PUMA, p53-up-regulated modulator of apoptosis; PIDD, p53-induced protein with death domain; NF-κB, nuclear factor κB; ROS, reactive oxygen species; CDK, cyclin-dependent kinase; ER, endoplasmic reticulum; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; mt, mitochondrial; hsp, heat shock protein; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad-3-related; DNA-PK, DNA-dependent protein kinase; ChK, checkpoint kinase.

cause of cell injury and death during chemotherapy, whereas p53 is considered to be a central mediator of the DNA damage response (Siddik, 2003; Wang and Lippard, 2005; Cepeda et al., 2007).

Despite the recognition of the role of p53 in cisplatin-induced cell death in cancer cells, whether it is critically involved in cisplatin nephrotoxicity was not determined until very recently. Nevertheless, research during the last few years has significantly advanced our understanding in this area. This review aims to: 1) summarize the recent evidence for the involvement of p53 in cisplatin nephrotoxicity; 2) dissect the signaling pathways upstream and downstream of p53 under the pathological condition; and 3) analyze the renoprotective strategy of targeting p53 during cisplatin-based cancer therapy.

Evidence for the Involvement of p53 in Cisplatin Nephrotoxicity

Increased level of nuclear p53 in the rat kidneys following cisplatin injection was first shown by Megyesi (1996). Five years later, using a similar in vivo model, Miyaji et al. (2001) reported a rapid nuclear accumulation of p53 in the outer medulla in response to cisplatin. Up-regulated p53 protein levels after cisplatin exposure were also shown in some in vitro models, including cultured mouse proximal tubular cells (Price et al., 2004), porcine proximal tubular cells (Xiao et al., 2003), and kidney slices from rat and human (Vickers et al., 2004).

Despite those findings, the role of p53 in cisplatin nephrotoxicity was not suggested until the study by Cummings and Schnellmann (2002). Using cultured rabbit proximal tubular cells, Cummings and Schnellmann (2002) detected p53 increase in the nucleus after 4 h of cisplatin treatment, preceding both caspase 3 activation and apoptosis. Notably, pifithrin-α, a pharmacological inhibitor of p53, partially suppressed caspase 3 activation and protected the cells from cisplatin-induced apoptosis. These pharmacological data suggested that p53 may play a role in cisplatin-induced renal tubular cell apoptosis (Cummings and Schnellmann, 2002).

Our recent work has further confirmed and extended the involvement of p53 in cisplatin nephrotoxicity using in vitro and in vivo models (Jiang et al., 2004; Wei et al., 2007). In cultured rat proximal tubular cells, p53 was shown to be rapidly phosphorylated and up-regulated after cisplatin treatment, before the initiation of cell apoptosis. The p53 activation was not inhibited either by carbobenzoxy-Val-Ala-Asp-fluoromethyl ketone or by Bcl-2, although both suppressed cisplatin-induced cell apoptosis, indicating that p53 activation was not a consequence of cell death. Pifithrin-α blocked p53 activation and, in turn, attenuated caspase activation and apoptosis. The results suggested that p53 activation may be an early signal for cisplatin-induced renal tubular cell apoptosis. Notably, cisplatin-induced apoptosis was also inhibited by a dominant-negative mutant of p53 that had diminished transcriptional activity due to a point mutation in the DNA binding site, further demonstrating that the proapoptotic effects of p53 in response to cisplatin may largely depend on its transcriptional activity toward target genes (Jiang et al., 2004).

In mice, cisplatin treatment induced p53 phosphorylation and accumulation mainly in the cell nuclei of cortex and outer medulla of the kidneys, accompanied by renal dysfunction and tissue damage. Costaining with fluorescein isothiocyanate-labeled proximal or distal tubule-specific lectins, phaseolus vulgaris agglutinin or peanut agglutinin, indicated that p53 was activated in both proximal and distal tubular cells, which are recognized as the primary sites of cisplatin injury. Notably, p53 activation in both proximal and distal tubules was partially colocalized with the apoptotic cells. Again, pifithrin-α suppressed cisplatin-induced p53 activation in the kidneys and ameliorated the damages to renal function and histology. Furthermore, cisplatin-induced renal failure was abrogated in p53 knockout mice, and the proximal tubular cells isolated from p53-deficient mice showed less apoptosis and better survival than their wild-type counterparts after cisplatin exposure (Wei et al., 2007).

It is worth pointing out that the spatial analysis of p53 in this study demonstrated the in vivo relationship between p53 activation and tubular cell apoptosis in response to cisplatin. First, p53 induction was detected in the cell nuclei of renal cortex and outer medulla. Second, staining of p53 and specific tubular cell markers showed p53 activation in both proximal and distal tubules, with the main site at the proximal tubules. Moreover, cisplatin-induced apoptosis was also shown mainly in proximal tubules. Importantly, quantitative analysis of the colocalization of p53-positive and terminal deoxynucleotidyl transferase-UTP nick end labeling-positive cells indicated that around 50% apoptotic cells had p53 induction, further supporting the in vivo association of p53 activation with renal tubular cell apoptosis (Wei et al., 2007). Together, these in vitro and in vivo results have demonstrated compelling evidence for the involvement of p53 in cisplatin nephrotoxicity.

Transcriptional Regulation of Cisplatin Nephrotoxicity by p53

A primary mechanism by which p53 mediates apoptosis is through transcriptional activation and repression of target genes whose promoters contain p53-binding sites (May and May, 1999; Vousden and Lu, 2002; Oren, 2003). Research during recent years has identified a number of apoptotic genes that are transcriptional targets of p53 (Vousden and Lu, 2002; Harris and Levine, 2005). These genes may subsequently participate in apoptosis via multiple pathways (El-Deiry, 2003; Slee et al., 2004; Yu and Zhang, 2005). Interestingly, several such genes have been shown to be regulated by p53 during cisplatin nephrotoxicity (Fig. 1).

PUMA

PUMA was identified as an important p53-responsive proapoptotic gene during genotoxic stress in cancer cells (Nakano and Vousden, 2001; Yu et al., 2001, 2003; Jeffers et al., 2003). Our recent work has demonstrated PUMA induction in experimental model of cisplatin nephrotoxicity (Jiang et al., 2006). It was shown that PUMA-α, but not other isoforms, was remarkably induced by cisplatin both in vitro in cultured proximal tubular cells and in vivo in mouse kidneys. This induction was dependent on p53 because it was inhibited by pifithrin-α and dominant-negative p53 and was also abrogated in p53-null mice. Moreover, cisplatin-induced

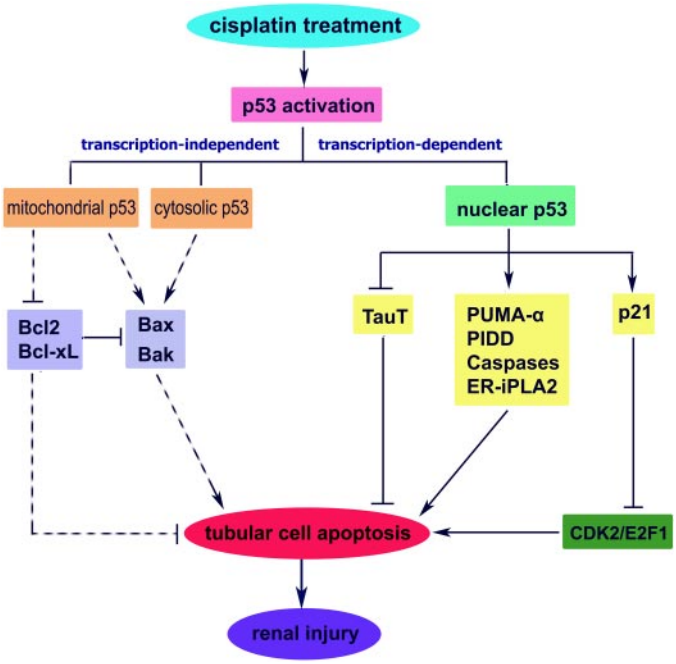

Fig. 1. p53 signaling pathways leading to tubular cell apoptosis during cisplatin nephrotoxicity. p53 is activated in response to cisplatin treatment. By transcriptional regulation, nuclear p53 may activate proapoptotic genes, including PUMA-α, PIDD, caspases, and ER-iPLA$_2$, and repress antiapoptotic genes, such as p21 and taurine transporter (TauT). In addition, p53 may also induce apoptosis by transcription-independent mechanisms via interactions with Bcl-2 family proteins in mitochondria and/or cytosol. Solid lines, supported by experimental data; dashed lines, to be tested.

renal tubular cell apoptosis was suppressed under these conditions. Consistently, cisplatin-induced apoptosis was ameliorated in PUMA-α knockout cells. Mechanistically, it was shown that PUMA-α accumulated in mitochondria after induction where it interacted with Bcl-xL to free proapoptotic molecules such as Bax. As a consequence, Bax was activated to permeabilize the mitochondrial membrane and release cytochrome *c*, leading to caspase activation and apoptosis. Thus, from both in vitro and in vivo experiments, these results have suggested the involvement of PUMA-α in p53-mediated renal cell apoptosis during cisplatin nephrotoxicity (Jiang et al., 2006).

PIDD

PIDD was first identified as a p53-regulated protein in mouse erythroleukemia cells and shown to promote apoptosis (Lin et al., 2000). A following study (Tinel and Tschopp, 2004) demonstrated that PIDD can activate caspase 2 via the formation of a protein complex called "PIDDosome" in response to genotoxic stress. In cultured proximal tubular cells, PIDD was shown to mediate cisplatin-induced apoptosis (Seth et al., 2005). In these cells, PIDD was induced by cisplatin, and the induction was attenuated by pifithrin-α and p53 small interfering RNA, suggesting a p53-dependent mechanism. After induction, PIDD activated caspase 2, which in turn induced mitochondrial release of apoptosis-inducing factor, resulting in chromatin condensation, nuclear DNA degradation, and tubular cell apoptosis (Seth et al., 2005).

Caspases

Transcriptional regulation of caspases by p53 has been discovered recently. It has been reported that caspases 1, 6, 7, and 10, with the p53-specific binding elements in their promoter regions, can be activated by p53 in a transcription-dependent fashion (Gupta et al., 2001; MacLachlan and El-Deiry, 2002; Rikhof et al., 2003; Joshi et al., 2007). Consistently, a very recent study (Yang et al., 2007) has suggested p53-dependent transactivation of caspases 6 and 7 in cisplatin nephrotoxicity and their significance to renal injury. Using both in vitro and in vivo model of cisplatin-induced acute kidney injury, the study first identified caspases 6 and 7 as the transcriptional targets of p53 and characterized the p53-binding sites in the genomic sequences of the two enzymes. By sequence-specific DNA binding, cisplatin-induced p53 or p53 overexpression increased the expression levels of caspases 6 and 7 as well as their activities in renal tubular cells and kidney cortex, whereas inhibition of p53 by pifithrin-α or p53 deficiency blocked the activation of both executioner caspases and protected the tubular cells from cisplatin-induced apoptosis, renal tissue damage, and renal dysfunction (Yang et al., 2007).

p21

In parallel with apoptosis, cell-cycle arrest accounts for another important function of p53 in response to DNA damage. Of the numerous p53 transcriptional targets identified so far, p21, a CDK inhibitor, stands out as a well recognized principal mediator of cell-cycle arrest. Interestingly, the role of p21 in cisplatin nephrotoxicity may not be limited to cell-cycle arrest but extends to apoptosis regulation. Price and colleagues (Megyesi et al., 1996) first reported a drastic p21 induction during cisplatin nephrotoxicity. Notably, the induction was largely but not completely abrogated in p53-deficient mice, indicating the presence of both p53-dependent and -independent mechanisms. Further investigation showed that, compared with their wild-type littermates, p21-null mice were highly sensitive to cisplatin-induced renal injury, with rapid and widespread tubular cell death, severe renal tissue damage, reduced survival, and increased mortality (Megyesi et al., 1998). Lack of functional p21 in primary cultures of mouse proximal tubular cells also augmented caspase-independent apoptosis induced by cisplatin (Nowak et al., 2003). Inhibition of p21 by antisense oligodeoxynucleotides significantly aggravated proximal tubule necrosis in cisplatin-treated rats (Zhou et al., 2006). Together, these observations have suggested that p21 induction is a renoprotective response during cisplatin nephrotoxicity. This inference is further supported by the protective effects of p21 overexpression in mouse proximal tubular cells (Price et al., 2004).

Mechanistically, using deletion mutants, a recent study has determined the functional domains of p21 that are responsible for its cytoprotective actions (Yu et al., 2005). A region of amino acids 38 to 91 at the N-terminal of p21, which contains the CDK2 binding moiety, was localized and identified (Yu et al., 2005). Further studies have suggested the role of CDK2 and its downstream effector E2F1 in both in vitro and in vivo models of cisplatin nephrotoxicity (Price et al., 2006; Yu et al., 2007). Therefore, these data demonstrated that through the functional domain binding to CDK2,

p21 inhibits CDK2 activity and protects the tubular cells from cisplatin-induced apoptosis (Yu et al., 2005; Price et al., 2006). A balance between p21 and CDK2 may therefore be a determining factor in cisplatin nephrotoxicity (Pabla and Dong, 2008).

Other Genes

Taurine transporter, an antiapoptotic gene with p53 binding site within its promoter, was shown to be down-regulated by cisplatin through a p53-dependent pathway, which contributed to subsequent renal cell apoptosis (Han and Chesney, 2005). ER-iPLA$_2$, an ER-associated protein, may regulate p53 signaling at different levels. In cancer cell lines, inhibition of ER-iPLA$_2$ induced growth arrest and probably apoptosis via p53-dependent and -independent mechanisms (Zhang et al., 2006; Sun et al., 2008); however, in renal tubular cells, ER-iPLA$_2$ was demonstrated to participate in cisplatin-induced apoptosis downstream of p53 and upstream of caspase 3 (Cummings et al., 2004). Thus, p53 may simultaneously induce cell death and survival signals during cisplatin nephrotoxicity through transactivation of different sets of genes. Apparently, the proapoptotic role that p53 plays seems to be dominant during cisplatin nephrotoxicity, as p53 inhibition or deficiency significantly reduces tubular cell apoptosis and prevents renal tissue damage and renal failure.

Transcription-Independent Regulation of Cisplatin Nephrotoxicity by p53?

Although transcriptional regulation by p53 has a major role in its proapoptotic action, recent evidence has also revealed the coexistence of a transcription-independent death pathway (Manfredi, 2003; Moll et al., 2005; Schuler and Green, 2005). Although it is less understood how p53 mediate apoptosis in the absence of transcription, two different mechanisms have been described, each of which was assigned to a specific p53 localization, either in cytosol or at the mitochondria (Fig. 1). In a series of in vitro and in vivo studies, a fraction of induced p53 was shown to translocate to mitochondria where it interacted with antiapoptotic Bcl-2 and Bcl-xL to liberate proapoptotic Bak and Bax or competed for Bak interaction with myeloid cell leukemia factor 1 (Mcl-1) to directly activate Bak, leading to the burst of apoptosis cascade (Dumont et al., 2003; Mihara et al., 2003; Erster et al., 2004; Leu et al., 2004). On the other hand, assays using immunopurified endogenous p53 to coincubate with recombinant Bax and isolated mitochondria or synthetic liposome have provided evidence that cytosolic p53 may also directly activate Bax without involving other protein synthesis or active transcription, leading to mitochondria leakage and subsequent engagement of apoptosis program (Chipuk et al., 2003, 2004). This inference is further confirmed by the experiments performed in mouse fibroblasts (Speidel et al., 2006). Apparently, both transcription-independent mechanisms eventually converge at the mitochondria and significantly synergize the transcription-dependent regulation of p53 in the nucleus to amplify its apoptotic potency (Fig. 1).

p53 translocation to mitochondria is an interesting observation with potential implications. First, it occurs rapidly, preceding the early collapse of mitochondrial membrane potential, cytochrome *c* release. and caspase 3 activation, on one hand, and the induction of p53 transcriptional targets, on the other hand (Marchenko et al., 2000; Erster et al., 2004). Second, this translocation is specific for p53-dependent apoptosis but not for p53-independent apoptosis or p53-mediated cell-cycle arrest (Marchenko et al., 2000). Third, the majority of p53 localizes at the mitochondrial outer membrane, whereas a small fraction is found in the matrix (Marchenko et al., 2000; Sansome et al., 2001). Moreover, p53 mitochondrial translocation seems to be a universal p53 response that takes place in a variety of malignant and nonmalignant cells and in normal tissues upon cellular stress (Moll et al., 2005). Further studies have investigated the possible mechanism of p53 delivery to mitochondria. mt hsp70 (also called Grp75) and mt hsp60, two key mitochondrial import motors that pull and trap proteins before they get into final mitochondria destination (Lill and Neupert, 1996; Pfanner and Meijer, 1997), were shown to interact with p53, suggesting a transient import step that involves mt hsp70 and mt hsp60 for p53 mitochondrial migration (Marchenko et al., 2000; Dumont et al., 2003). A recent study using various manipulations that enhance or diminish p53 ubiquitylation has demonstrated that Mdm2-mediated monoubiquitylation promotes p53 mitochondrial translocation and subsequent apoptosis (Marchenko et al., 2007).

Could cisplatin induce p53 translocation to mitochondria? Apparently the answer is yes, at least for some cancer cell lines. Cisplatin induced a rapid p53 translocation to mitochondria in human colon carcinoma HCT116 cells (Bragado et al., 2007). In KB human epidermoid cancer cells, a fraction of p53 was shown to localize to mitochondria and interact with mitochondrial transcription factor A after cisplatin exposure, resulting in the activation of mitochondrial transcription factor A binding to cisplatin-damaged DNA that might play an important role in apoptosis (Yoshida et al., 2003). Furthermore, Sayan et al. (2006) have suggested a caspase-dependent cleavage of p53 in response to cisplatin in a variety of cultured tumor cells. Importantly, the p53-cleaved fragments were shown to translocate to mitochondria and induce mitochondrial membrane depolarization. Recent work by Yang et al. (2006) has shown a mitochondrial p53 accumulation and its relevance to subsequent mitochondrial release of apoptotic proteins and apoptosis in chemosensitive but not resistant ovarian cancer cells treated by cisplatin, further demonstrating a direct p53-mediated mitochondrial death program. Despite these findings, whether p53 translocates to mitochondria during cisplatin nephrotoxicity and participates in tubular cell apoptosis has not been reported. Certainly, this can be an important area of study in the future.

Upstream Signaling Responsible for p53 Activation in Cisplatin Nephrotoxicity

DNA Damage Response

Although the signaling pathways leading to p53 activation in response to cisplatin are largely unclear, cisplatin-induced DNA damage is thought to be a major and direct trigger for p53 activation (Siddik, 2003; Norbury and Zhivotovsky, 2004). In general, this DNA damage can be sensed by a number of protein kinases that in turn stabilize p53 through post-translational modifications, leading to p53 activation

(Appella and Anderson, 2001; Siddik, 2003; Norbury and Zhivotovsky, 2004). The protein kinases that have been identified include ATM, ATR, DNA-PK, ChK, protein kinase C, CDK, mitogen-activated protein kinase, casein kinases I and II (Lakin and Jackson, 1999; Shiloh, 2003; Bode and Dong, 2004). Are these kinases involved in the DNA damage response and p53 activation during cisplatin nephrotoxicity? Our recent studies examined ATM, ATR, and DNA-PK, three major DNA damage-responsive protein kinases (Wang et al., 2006; Pabla et al., 2007). ATM was shown to be proteolytically cleaved in the late stage of cisplatin treatment in renal tubular cells. It is important to note that cleavage of ATM was mediated by caspases and appeared to inactivate its kinase activity toward p53 (Wang et al., 2006; Pabla et al., 2007). Similar inactivation of DNA-PK was also detected after cisplatin exposure (Pabla et al., 2007). In contrast, ATR was activated early during cisplatin treatment of renal tubular cells, shown as increased kinase activity and formation of the RAD9-RAD1-HUS1 (9-1-1) protein complex. The activated ATR accumulated in the nuclei and colocalized with histone H2AX to form nuclear foci at the site of DNA damage. Chk2, one of the two major checkpoint protein kinases that are downstream of ATR/ATM, was subsequently activated in an ATR-dependent manner. Inhibition of ATR or Chk2 either by dominant-negative mutants or by gene deficiencies attenuated cisplatin-induced p53 activation and cell apoptosis. Furthermore, in vivo experiments showed the activation of ATR and Chk2 in renal tissues after cisplatin treatment. Therefore, this study has demonstrated the first evidence for an early DNA damage response mediated by ATR and Chk2 during cisplatin nephrotoxicity and, importantly, a role for ATR/Chk2 signaling in cisplatin-induced p53 activation and tubular cell apoptosis (Fig. 2) (Pabla et al., 2007).

The DNA damage response is a highly complex event that is orchestrated by multiple proteins and signaling cascades generally including damage detection by sensors, signal relaying and amplification by transducers, and execution of DNA repair, cell-cycle arrest, and apoptosis by effectors (Norbury and Zhivotovsky, 2004). In the above study, the specificity of ATR activation during cisplatin treatment in renal tubular cells is of particular interest, although it is not completely clear why and how. It has been reported that different types of DNA damage can be sensed and signaled by independent molecular complexes (Harper and Elledge, 2007). Thus, the formation of a multiprotein complex by ATR but not ATM with the 9-1-1 complex raises the possibility that the specific ATR activation induced by cisplatin might be related to the unique complex recruitment and interaction of signaling proteins at the DNA damage sites (Pabla et al., 2007).

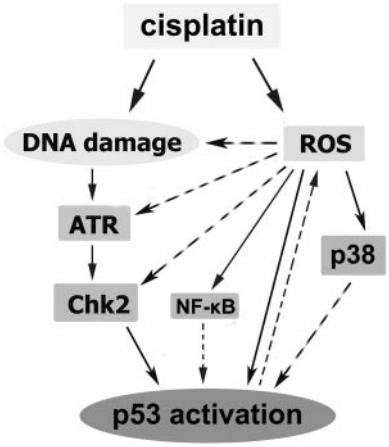

Fig. 2. Upstream signaling for p53 activation during cisplatin nephrotoxicity. Cisplatin treatment induces a rapid DNA damage response, leading to the activation of ATR, which further phosphorylates and activates Chk2. Both ATR and Chk2 can phosphorylate p53 for its activation. ROS may also contribute to p53 activation by multiple pathways. Solid lines, supported by experimental data; dashed lines, to be tested.

ROS

As discussed, platinum-DNA adduct formation is the primary and classic mechanism for cisplatin-induced DNA damage; however, other signals induced by cisplatin may also trigger this cytotoxic event taking into account the high reactivity of the drug. Oxidative stress may be one of such signals. Oxidative stress has been implicated in cisplatin nephrotoxicity for years (Baliga et al., 1999; Nath and Norby, 2000; Taguchi et al., 2005). Cisplatin may induce the generation of various ROS through inactivation of cellular antioxidant system, disruption of mitochondrial respiratory chain, or interaction with microsomal cytochrome P450 (Pabla and Dong, 2008). Highly reactive ROS appear to target multiple cellular components, such as lipids, proteins, and DNA, and activate multiple signaling pathways (Allen and Tresini, 2000; Martindale and Holbrook, 2002). The involvement of ROS in p53 activation during cisplatin nephrotoxicity was further suggested by our recent study (Jiang et al., 2007). ROS, particularly hydroxyl radicals, accumulated rapidly following cisplatin incubation of renal tubular cells, correlating with the early p53 activation. Suppression of hydroxyl radical formation by ROS scavengers, dimethylthiourea and *N*-acetyl-cysteine, attenuated p53 activation, protected the tubular cells from cisplatin-induced apoptosis, and preserved renal tissue histology and function in mice (Jiang et al., 2007).

Despite the contribution of hydroxyl radicals to early p53 activation during cisplatin nephrotoxicity, the underlying mechanism is still unclear. Under the circumstance that ROS induce DNA damage, it would be interesting to investigate the DNA damage-responsive pathway, such as ATR/Chk2 and their possible regulation by hydroxyl radicals during cisplatin nephrotoxicity (Fig. 2). In addition to ATR/Chk2, several other protein kinases, including protein kinase C, extracellular signal-regulated kinase, c-JUN NH_2-terminal kinase, and p38 have been implicated in p53 activation during genotoxic stress (Appella and Anderson, 2001; Bode and Dong, 2004). Meanwhile, these protein kinases have also been shown to participate in cisplatin-induced renal injury (Nowak, 2002; Arany et al., 2004; Sheikh-Hamad et al., 2004; Jo et al., 2005; Kim et al., 2005; Ramesh and Reeves, 2005; Mishima et al., 2006; Francescato et al., 2007a). Notably, Ramesh and Reeves (2005) have revealed an activation of p38 by hydroxyl radicals during cisplatin nephrotoxicity. Based on these observations, it would be interesting to determine whether these protein kinases (e.g. p38) relay ROS signaling to p53 during cisplatin nephrotoxicity (Fig. 2).

In addition, NF-κB could be another molecule that possibly couples ROS with p53 to regulate cisplatin nephrotoxicity. NF-κB is a family of transcription factors that regulate the expression of a number of genes involved in diverse cellular responses, such as immune reaction, inflammation, cell proliferation, and apoptosis (Pahl, 1999). The activation of

NF-κB and its role in cisplatin-induced renal injury have been suggested in a series of in vivo and in vitro studies (Ramesh and Reeves, 2004; Li et al., 2005; Lee et al., 2006a,b; Francescato et al., 2007b; Luo et al., 2008). Notably, cisplatin-induced oxidative stress appears to be a potent activator of NF-κB because the antioxidants can attenuate ROS generation and NF-κB activation and thus protect the renal cells against cisplatin injury (Lee et al., 2006b; Luo et al., 2008). Mechanistically, NF-κB-mediated tumor necrosis factor-α induction and inflammation have been attributed to the renal cell death (Schrier, 2002), whereas it is unclear whether p53 participates in the ROS/NF-κB signaling during cisplatin nephrotoxicity. In neurons exposed to DNA damage, the activated NF-κB has been shown to act upstream of p53 to modulate its transcription activity and induce neuron cell apoptosis (Aleyasin et al., 2004). Thus, future research is needed to address the role of NF-κB as a modulator of p53 to regulate cisplatin nephrotoxicity (Fig. 2). It is worth noting that NF-κB activation after cisplatin exposure has a distinct role in cancer cells. The involvement of NF-κB in resistance of cancer cells to platinum-based chemotherapy has been clearly demonstrated, and inhibition of NF-κB by different methods has been shown to sensitize cancer cells to the drug (Lagunas and Melendez-Zajgla, 2008). In this case, investigation on NF-κB-dependent p53 regulation of cisplatin nephrotoxicity would be important to the development of renoprotective strategy during cancer therapy.

A direct redox modification named *S*-glutathionylation has been suggested recently to contribute to p53 regulation by cisplatin-induced ROS in tumor cells (Velu et al., 2007). The glutathionylation was shown to locate at the cysteine sites of p53 DNA binding domain and inhibit its DNA binding activity, leading to p53 inactivation that may present a defensive response for the tumor cells (Velu et al., 2007). Other studies have proposed that different structures or sites of redox modification might be related to p53 target gene selection and outcomes of cellular response (Liu et al., 2008). Apparently, it is important to examine the possible redox modifications of p53 and its effects on cisplatin nephrotoxicity.

Instead of being an upstream signal that triggers p53 activation, ROS has also been shown to be generated downstream of p53 through transcriptional modulation of redox genes, including PIG3 (p53-induced gene), manganese superoxide dismutase, and glutathione peroxidase (Martindale and Holbrook, 2002). In addition, recent work by Matoba et al. (2006) has demonstrated the transactivation of synthesis of cytochrome *c* oxidase 2 by p53 and its effects on mitochondrial aerobic respiration and ROS formation (Matoba et al., 2006). Therefore, oxidant-induced p53 may further increase the level of ROS, leading to a positive feedback loop that strongly enhances p53 activation and apoptosis (Fig. 2). Such a feedback has been proposed in cisplatin-induced HCT116 cell apoptosis (Bragado et al., 2007). Further study is required to determine the interaction and cooperation between ROS and p53 signals in cisplatin renal injury.

Is p53 a Strategic Target for Renoprotection during Cisplatin-Based Cancer Therapy?

The involvement of p53 in cisplatin nephrotoxicity indicates that inhibition of p53 may protect kidneys during cisplatin-based cancer therapy. The discovery and development of pharmacological inhibitors of p53 further supports the feasibility of this renoprotective strategy (Komarov et al., 1999; Gudkov and Komarova, 2005; Strom et al., 2006). However, p53 is a well recognized tumor suppressor. Moreover, cancer therapy may depend, in part, on p53-mediated cell killing in tumors. These considerations raise important questions. Is targeting p53 a useful strategy for renoprotection during cisplatin-based cancer therapy? Will p53 inhibition block the therapeutic effects in tumors or cancer cells? Will p53 inhibition induce cancer progression or new tumors? Although we do not have a clear answer, existing laboratory and clinical findings may help address these questions to some extent. First, p53 is lost or mutated in over 50% of tumors; yet, cisplatin is effective in treating them, indicating p53-independent anticancer mechanisms (Siddik, 2003; Wang and Lippard, 2005; Cepeda et al., 2007). In these tumors, protection of kidneys by p53 inhibitors is not expected to diminish the therapeutic effects of cisplatin in the tumors. Second, in tumors where p53 is the key to cancer cell killing, it is possible to specifically deliver p53 antagonists to kidneys or renal tissues. Such approaches have been reported and are being developed (Tomasoni and Benigni, 2004; Tomita et al., 2004). With specific kidney delivery of the pharmacological or genetic inhibitors of p53, it is expected that cisplatin-induced renal injury may be abrogated, whereas antitumor activity of the drug is not affected. Third, in some tumor types, wild-type p53 has been shown to be responsible for chemotherapy resistance and act as a survival factor. Under these circumstances, p53 inhibition for renoprotection may even sensitize the tumor cells to the anticancer drug and promote therapeutic efficacy (Gudkov and Komarova, 2005). Finally, unlike p53-deficient mice with cancer predisposition (Donehower et al., 1992), it has been shown that pharmacologic blockade of p53 was not associated with a high incidence of new cancer development, indicating that the temporary and reversible p53 suppression during cancer therapy can be relatively safe (Komarov et al., 1999).

Therefore, depending on tumor types and status, it is possible to inhibit p53 for renoprotection during cisplatin therapy. Certainly, this possibility should be further investigated by using tumor-bearing animal models.

Perspectives and Conclusions

Renal tubular cell apoptosis during cisplatin nephrotoxicity involves multiple signaling pathways (Arany and Safirstein, 2003; Hanigan and Devarajan, 2003; Pabla and Dong, 2008). Here we have discussed the major apoptotic pathways involving p53. In this signaling cascade, cisplatin-induced DNA damage is considered to be an important trigger of p53 activation. Accordingly, as an integral part of cellular response to genotoxic stress, p53 couples DNA damage response to cell apoptosis. Of note, cisplatin may also react with and induce damage to other cellular components or structures, including membrane phospholipids, cytoskeletal microfilaments, thiol-containing biomolecules, and cytoplasmic proteins, resulting in cell death independent of DNA damage (Cepeda et al., 2007). This possibility is supported by the latest work by Yu et al. (2008). It was shown that enucleated mouse renal tubular cells underwent cytoplasmic changes of apoptosis during cisplatin incubation. Thus, cisplatin can induce cell injury and death in the absence of nucleus or DNA damage. Does it suggest that DNA damage

is irrelevant or unimportant in cisplatin nephrotoxicity? The answer is no because, in reality, each tubular cell has a nucleus and DNA damage response occurs during cisplatin treatment. Therefore, it is likely that the nuclear and cytoplasmic mechanisms cooperate to orchestrate an apoptosis program. Currently, it is unknown how cisplatin activates apoptotic signaling in cytoplasm in the absence of the nucleus or DNA damage. In addition, it is unclear whether and how cytoplasmic and nuclear signaling pathways are integrated.

Can p53 participate in the signaling integration? A recent study has suggested a cooperation of transcription-dependent and -independent proapoptotic effects of p53 during genotoxic stress (Chipuk et al., 2005). It was shown that, after UV exposure, p53 induction in the nucleus transactivated PUMA-α, which subsequently released p53 that was sequestered by Bcl-xL in cytosol. As a result, p53 directly activated Bax in the cytosol to induce apoptosis (Chipuk et al., 2005). This is certainly an attractive scenario that should be thoroughly evaluated in model systems of cisplatin nephrotoxicity.

As discussed, p53 signaling plays an important role in cisplatin nephrotoxicity. However, neither pharmacologic inhibitor nor genetic deficiency of p53 could completely diminish renal cell apoptosis (Cummings and Schnellmann, 2002; Jiang et al., 2004; Wei et al., 2007), indicating the presence of p53-independent mechanisms. Therapeutically, considering the complexity of cisplatin nephrotoxicity and possible integration of multiple signaling pathways, combinational strategies by simultaneously targeting several injury pathways may enhance the renoprotective efficacy (Pabla and Dong, 2008). Such strategies should be thoroughly evaluated in cultured cells and animals, particularly, tumor-bearing animals.

In conclusion, recent studies have demonstrated compelling evidence for a pathological role of p53 in cisplatin nephrotoxicity. The signaling pathways upstream and downstream of p53 are being elucidated. In combination with other approaches, targeting p53 may offer a clinically applicable strategy for renoprotection during cisplatin-based cancer therapy.

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