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Cisplatin: mode of cytotoxic action and molecular basis of resistance

Zahid H Siddik*[,1]

[1]*Department of Experimental Therapeutics, Unit 104, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009, USA*

Cisplatin is one of the most potent antitumor agents known, displaying clinical activity against a wide variety of solid tumors. Its cytotoxic mode of action is mediated by its interaction with DNA to form DNA adducts, primarily intrastrand crosslink adducts, which activate several signal transduction pathways, including those involving ATR, p53, p73, and MAPK, and culminate in the activation of apoptosis. DNA damage-mediated apoptotic signals, however, can be attenuated, and the resistance that ensues is a major limitation of cisplatin-based chemotherapy. The mechanisms responsible for cisplatin resistance are several, and contribute to the multifactorial nature of the problem. Resistance mechanisms that limit the extent of DNA damage include reduced drug uptake, increased drug inactivation, and increased DNA adduct repair. Origins of these pharmacologic-based mechanisms, however, are at the molecular level. Mechanisms that inhibit propagation of the DNA damage signal to the apoptotic machinery include loss of damage recognition, overexpression of HER-2/neu, activation of the PI3-K/Akt (also known as PI3-K/PKB) pathway, loss of p53 function, overexpression of antiapoptotic bcl-2, and interference in caspase activation. The molecular signature defining the resistant phenotype varies between tumors, and the number of resistance mechanisms activated in response to selection pressures dictates the overall extent of cisplatin resistance.
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Introduction

Since its introduction into clinical trials, cisplatin (*cis*-diammine-dichloro-platinum^{II}) has had a major impact in cancer medicine, changing the course of therapeutic management of several tumors, such as those of the ovary, testes, and the head and neck (Prestayko *et al*., 1979). Almost 30 years after its clinical benefits were first recognized, studies still continue in an effort to understand exactly how cisplatin works. There is no doubt, however, that DNA is the primary target of cisplatin (Roberts and Pera Jr, 1983), but still there are wide gaps in our fuller appreciation of the process that translates cisplatin-induced DNA damage into its characteristic drug-mediated cellular effects, namely, inhibition of DNA synthesis, suppression of RNA transcription, effects on the cell cycle, and the therapeutically beneficial process of apoptosis. An understanding of the mode of action is indeed desirable in refining therapeutic approaches that further enhance the antitumor activity of the platinum drug. This understanding is also critical for elucidating mechanisms underlying the drug-resistant phenotype, which radically limits the clinical utility of cisplatin. An excellent example to highlight this limitation is with ovarian cancer, which generally responds well to cisplatin-based therapy. Unfortunately, the initial response rate of up to 70% is not durable, and results in a 5-year patient survival rate of only 15–20%, primarily as tumors become resistant to therapy (Ozols, 1991). In an alternative example with small cell lung cancer, the relapse rate can be as high as 95% (Giaccone, 2000). The onset of resistance creates a further therapeutic complication in that tumors failing to respond to cisplatin are cross-resistant to diverse unrelated antitumor drugs (Ozols, 1992). This suggests that cisplatin and the other agents likely share common mechanisms of resistance. In this respect, it is noteworthy that cisplatin-resistant tumors are fully crossresistant to the platinum analog carboplatin (Gore *et al*., 1989; Eisenhauer *et al*., 1990). Thus, to circumvent resistance, alternative DNA damage-signaling pathways need to be evoked, as has been demonstrated experimentally with ionizing radiation and the platinum analog DACH-acetato-Pt (Hagopian *et al*., 1999; Siddik *et al*., 1999). It is indeed likely that the demonstration of increased sensitivity of resistant cells to distinct platinum drugs, such as ZD0473 (Kelland *et al*., 1999) and oxaliplatin (Faivre *et al*., 1999) may in part reflect activation of independent pathways. Utilization of such agents in comparative investigations may prove to be invaluable for unraveling fully the mechanism of cisplatin resistance.

Mode of drug action

The pathways involved in cisplatin-induced cytotoxicity are summarized in Figure 1, and described in detail in the following sections.

*Correspondence: ZH Siddik; E-mail: zsiddik@mdanderson.org

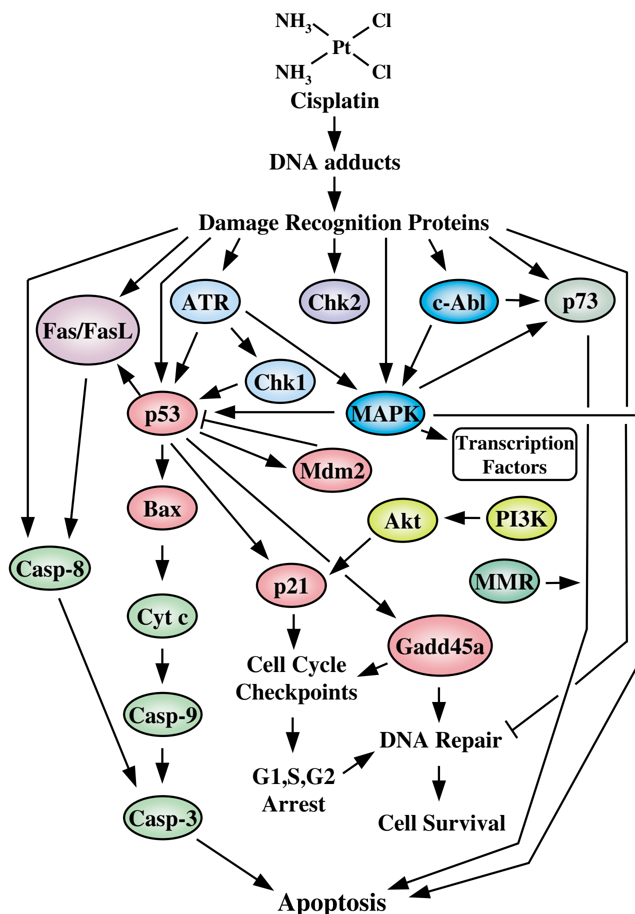

Figure 1 An overview of pathways involved in mediating cisplatin-induced cellular effects. Cell death or cell survival will depend on the relative intensity of the signals generated and the crosstalk between the pathways involved. Some of the signaling discussed in the text has been omitted for clarity

Drug reactivity

Cisplatin is a neutral inorganic, square planar complex that reacts with DNA to induce its characteristic biological effects, which culminate in either repair of the DNA damage and cell survival or activation of the irreversible apoptotic program. However, for interaction to occur with DNA, the neutral cisplatin has to be activated through a series of spontaneous aquation reactions, which involve the sequential replacement of the *cis*-chloro ligands of cisplatin with water molecules (el Khateeb *et al*., 1999; Kelland, 2000). The monoaquated form is recognized as a highly reactive species, but its formation is rate limiting in the interaction with many endogenous nucleophiles, such as glutathione (GSH), methionine, metallothionein, and protein. Thus, when cisplatin enters cells, it is potentially vulnerable to cytoplasmic inactivation by these and other intracellular components.

DNA adducts and damage recognition

The cytotoxicity of cisplatin is primarily ascribed to its interaction with nucleophilic N7-sites of purine bases in DNA to form DNA–protein and DNA–DNA interstrand and intrastrand crosslinks (Eastman, 1987b). However, evidence strongly favors intrastrand adducts as lesions largely responsible for the cytotoxic action (Pinto and Lippard, 1985). This is consistent with the knowledge that 1,2-intrastrand ApG and GpG crosslinks are the major forms of DNA adducts, accounting for 85–90% of total lesions (Kelland, 1993). A similar preponderance of these intrastrand adducts has also been reported in cultured cells for the structurally distinct analog DACH-sulfato-platinum^{II} (Jennerwein *et al*., 1989). This eliminates the possibility that the favorable cytotoxicity of such analogs against cisplatin-resistant tumor cells (Eastman, 1987a) is due to a qualitative or quantitative difference in DNA bases that are targeted.

Since intrastrand DNA adducts comprise the bulk of cisplatin-induced nuclear lesions, it is not surprising that a linear correlation has been found between gross levels of platinum bound to DNA and the extent of cytotoxicity (Fraval and Roberts, 1979; Roberts and Fraval, 1980). Although cisplatin affects DNA replication, no correlation exists between inhibition of DNA synthesis and cytotoxicity (Sorenson and Eastman, 1988). It is only recently that we have come to understand better the sequence of events extending from the formation of DNA adducts to the completion of the cytotoxic process, namely apoptosis. This sequence is likely initiated or facilitated following the recognition of DNA damage by over 20 individual candidate proteins, which bind to physical distortions in the DNA that are induced by the intrastrand platinum adducts (Bellon *et al*., 1991). These damage recognition proteins include the hMSH2 or hMutSα component of the mismatch repair (MMR) complex, the nonhistone chromosomal high-mobility group 1 and 2 (HMG1 and HMG2) proteins, the human RNA polymerase I transcription ‘upstream binding factor’ (hUBF), and the transcriptional factor ‘TATA binding protein’ (TBP) (Donahue *et al*., 1990; Fink *et al*., 1998; Chaney and Vaisman, 1999). Whether a single protein or combinations of these are involved in sensing the damage is not clear. What is interesting is that a few of the proteins, exemplified by MMR and HMG1, demonstrate greater preference for cisplatin adducts than for adducts induced by distinct platinum analogs, such as the clinically active oxaliplatin and JM216 (Fink *et al*., 1996; Chaney and Vaisman, 1999; Zdraveski *et al*., 2002).

Although the likely role of DNA damage recognition proteins is to transduce DNA damage signals to downstream effectors, their biological relevance may not be limited to this function alone. The HMG1 protein, for instance, has been implicated in promoting cytotoxicity by first interacting with the DNA adduct and then shielding it from repair (Huang *et al*., 1994). This action of HMG1 is supported by the finding that overexpression of this recognition protein by pre-exposure to estrogen sensitizes breast tumor cells to cisplatin (He *et al*., 2000). Similarly, hUBF and TBP are involved in the initiation of transcription by RNA

polymerase I, and it is feasible that cisplatin adducts sequester these factors at the damaged DNA sites, and prevent their participation in transcription (Jordan and Carmo-Fonseca, 2000). The resulting inhibition of transcription may itself serve as a trigger for transducing DNA damage signals. It appears reasonable to suggest, therefore, that each of the recognition proteins may initiate one or more specific events, so that DNA damage results in several seemingly unrelated biological effects. This is consistent with the understanding that adducts induced by cisplatin disrupt replication and transcriptional processes, but that such biological effects do not necessarily correlate directly with cell death (Jordan and Carmo-Fonseca, 2000). This can also be reconciled by the understanding that both pro-survival and pro-apoptotic signals are activated simultaneously following cisplatin exposure, and the relative intensity and/or duration of each is integrated downstream to determine the final fate of the cell.

Cell cycle checkpoints

The notion that cisplatin-induced DNA damage activates a number of pathways is borne out from several investigations. One of these pathways culminates in the activation of cell cycle checkpoints, which temporally induce a transient S-phase arrest, followed by inhibition of the Cdc2-cyclin A or B kinase to affect a durable G2/M arrest (Shi *et al*., 1994; Shapiro and Harper, 1999; He *et al*., 2001). Since the inhibitory effect of DNA adducts of cisplatin on the G1-phase cyclin-dependent kinases (CDKs) is a later event in the sequence of checkpoint activation (He *et al*., 2001), and likely facilitated by the Cdk4 inhibitor p16^{INK4A} (Shapiro *et al*., 1998), significant accumulation of cells in the G1 phase is seen infrequently, largely because cells remain trapped in G2/M. The relationship between cell cycle arrest and cytotoxicity is complex and not fully deciphered. If anything, cell cycle arrest is seen as inhibitory to the cytotoxic process, which is a conclusion that derives primarily from the demonstration that pharmacological abrogation of the G2/M checkpoint increases cellular sensitivity to cisplatin (Demarcq *et al*., 1994; O'Connor and Fan, 1996). This is consistent with the concept that cell cycle arrest, as a generally accepted consequence of DNA damage, is necessary to enable the nucleotide excision repair (NER) complex to remove the adducts and promote cell survival. Only when repair is incomplete, as would be the case when damage is extensive, will cells undergo apoptosis. Thus, repair is intimately linked to checkpoint activation and apoptosis, and it is interesting that all three processes are collectively associated with the tumor-suppressor p53 protein (Morgan and Kastan, 1997; Bullock and Fersht, 2001). It is evident that our understanding of cellular and molecular responses to DNA-damaging agents has increased substantially during the past few years, but many important questions remain, including how p53 senses the extent of DNA damage repair and, thereby, determines whether to permit the cell to survive or activate the apoptotic program.

Activation of p53 and MAPK

Although the mediation of p53 in the cellular toxic effects of cisplatin is a direct consequence of DNA damage, a number of events must first occur to induce and activate the p53 protein molecule. A known upstream event is activation of kinases that regulate the stability and transcriptional activity of the p53 tumor suppressor. Among the two kinases involved in checkpoint activation, namely ATM (ataxia telangiectasia mutated protein) and ATR (ATM- and Rad3-related protein), cisplatin preferentially activates ATR kinase (Damia *et al*., 2001; Zhao and Piwnica-Worms, 2001), which phosphorylates p53 at serine-15 to initiate activation of the p53 protein (Appella and Anderson, 2001). ATR also activates other downstream targets as a step toward further modification of p53 at additional sites. Thus, ATR-mediated activation of CHK1 kinase results in phosphorylation at serine-20 of p53 (Shieh *et al*., 2000). Interestingly, cisplatin also activates CHK2, which is a downstream target of ATM, but the effect of cisplatin on CHK2 appears to be independent of ATM (Damia *et al*., 2001). More recently, ATR has been linked to the activation of specific pathways of the mitogen-activated protein kinase (MAPK) cascade (Tang *et al*., 2002; Zhang *et al*., 2002), which phosphorylates p53 in a number of positions, including serine-15 (Persons *et al*., 2000) and threonine-81 (Appella and Anderson, 2001).

The involvement of the MAPK pathway in cisplatin's mode of action is of significant interest. The major MAPK subfamily members include the extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases (JNKs, also referred to as stress-activated protein kinase (SAPK)), and the p38 kinases. These MAPK members participate in integrating extracellular signals to regulate cell proliferation, differentiation, cell survival, and apoptosis (Dent and Grant, 2001). Studies by Wang *et al.* (2000) have demonstrated that all three kinase members are activated following exposure of tumor cells to cisplatin. These authors, however, suggest that ERK activation is the most critical for cisplatin-induced apoptosis, which is consistent with the demonstration that ERK activated by cisplatin contributes to p53 regulation by phosphorylating the tumor-suppressor protein at serine-15 (Persons *et al*., 2000). Furthermore, inhibition of the MEK–ERK pathway leads to cisplatin resistance (Yeh *et al*., 2002). Reports by others, however, are in direct contrast and suggest that activation of ERK and JNK MAPK cascades by cisplatin antagonizes apoptosis (Dent and Grant, 2001). It is possible that both effects mediated through MAPK are correct, and the apparent discrepancy may merely reflect differences in cell context or the extent of DNA damage. Thus, it may be premature at this stage to disassociate any MAPK subfamily members from the cytotoxic effects of cisplatin.

p53-dependent functions

Induction and/or activation of p53 is recognized as a prerequisite for its function as a sequence-specific transcription activator. Interestingly, HMG1 and HMG2 facilitate the binding of p53 to DNA to stimulate transactivation, and this enables HMG proteins to establish a direct link between damage recognition and activation of p53 function (Jayaraman *et al*., 1998). Several genes transactivated by p53 as a result of cisplatin exposure are associated with cell cycle arrest, DNA repair, and apoptosis, including CDK inhibitor *p21*$^{Waf1/Cip1}$, growth arrest and DNA damage-inducible *gadd45a* gene, and the pro-apoptotic *bax* gene (Delmastro *et al*., 1997; Hershberger *et al*., 2002). The p53 protein can also transactivate *mdm2*, which is a negative feedback regulator of p53 activity (Alarcon-Vargas and Ronai, 2002). With regard to repair, the Gadd45a protein associates with proliferating cell nuclear antigen (PCNA), enhances NER activity, and protects cells from cisplatin-induced cytotoxicity (Smith *et al*., 1994; Delmastro *et al*., 1997; Smith *et al*., 1997). However, when DNA damage exceeds a critical threshold, and presumably overwhelms cellular repair capacity, the net biological effect favors activation of apoptosis. This form of cell death is a complex, well-orchestrated process that begins with the translocation of the cisplatin-induced Bax from the cytosol to the mitochondria, where a cascade of events, involving the release of apoptogenic factors (such as cytochrome *c*) activates the caspase 9–caspase 3 pathway, and results in apoptosis (Wang *et al*., 2000; Makin *et al*., 2001). More specifically, the apoptotic process is regulated by the ratio between Bax and its opposing but closely related antiapoptotic counterpart Bcl-2. When Bax is induced by cisplatin, the Bax : Bcl-2 ratio increases and apoptosis ensues. However, cisplatin may also induce cleavage of Bcl-2, and either the resultant Bax-like cleaved product or the effective increase in the Bax : Bcl-2 ratio activates the apoptotic cascade (del Bello *et al*., 2001). Apoptosis induced by cisplatin also occurs through the Fas/FasL-activated caspase 8–caspase 3 pathway, which is facilitated by p53 function, but does not necessarily involve the mitochondria (Micheau *et al*., 1997; Muller *et al*., 1998). However, this pathway is not well understood as caspase 8 or apoptosis can be activated by cisplatin independent of Fas/FasL in some systems (Eischen *et al*., 1997; Ferreira *et al*., 2000).

Induction of apoptosis

Although the propensity of the reported data supports a facile role for p53 in cisplatin-induced apoptosis (Fan *et al*., 1994; Segal-Bendirdjian *et al*., 1998), there are several reports that deviate from this understanding. Fan *et al*. (1995) and Hawkins *et al*. (1996), for instance, have demonstrated that disruption of p53 function sensitizes tumor cells to the platinum drug, and do not make them resistant, as would be expected. It is useful to note that this counterintuitive finding is associated with tumor cells that appear to have an apoptotic dysfunction (Fan *et al*., 1995). How eliminating p53 function makes such cells more sensitive to cisplatin is unclear, but it is likely that cell cycle effects come into play, since sensitization to cisplatin is mediated through down-regulation of the p53-dependent *p21*$^{Waf1/Cip1}$ gene (Fan S *et al*., 1997). The increased sensitivity to cisplatin in such cases may be ascribed to a loss in the contributory role of p21$^{Waf1/Cip1}$ in G2/M arrest, resulting in premature entry into mitosis, with cell death being the final outcome. Such an effect is analogous to the observed sensitization of tumor cells to cisplatin by agents that abrogate the G2/M checkpoint (O'Connor and Fan, 1996). A further demonstration of the ability of cisplatin to induce cytotoxicity through a mechanism not involving p53 comes from the work of Gong *et al*. (1999), who reported that the protein product of a p53-related gene, *p73*, can also be induced by cisplatin to mediate apoptosis. Indeed, this group has demonstrated the coexistence of p53- and p73-dependent parallel apoptotic pathways for affecting cisplatin-induced cytotoxicity. Induction of p73-dependent apoptosis by cisplatin has two requirements: (1) drug-activated c-Abl tyrosine kinase and (2) cellular proficiency of the MMR complex, which, as with HMG1, links damage recognition to apoptotic signaling. c-Abl activated by cisplatin can also upregulates the MEKK–MKK–JNK pathway (Kharbanda *et al*., 2000), but the implied association between this specific MAPK pathway and p73 has been uncertain previously. However, the case for this association has been strengthened by recent evidence, which shows that activation of p73 by c-Abl also requires the activity of p38 as a representative of the MAPK subfamily member (Sanchez-Prieto *et al*., 2002).

Mechanism of resistance

The major goal of cancer chemotherapy is to commit tumor cells to apoptosis following exposure to antitumor agents. Although the inorganic drug cisplatin is a very potent inducer of apoptosis (Ormerod *et al*., 1996; Henkels and Turchi, 1997), resistance develops and is implied when tumor cells fail to undergo apoptosis at clinically relevant drug concentrations. This resistance can be acquired through chronic drug exposure or it can present itself as an intrinsic phenomenon. The exact level of cisplatin resistance in patients is difficult to define, but at least a twofold resistance is inferred from clinical studies, primarily since responses have been observed when the standard clinical dose of cisplatin is doubled in drug-intensive therapy protocols (Ozols *et al*., 1984, 1988; Schilder and Ozols, 1992). In general, resistance to cisplatin may be substantially greater, as judged from studies with tumor cell lines established from clinically refractory tumors, which require cytotoxic concentrations as much as 50–100-fold in excess of those needed for sensitive tumor cells (Hills *et al*., 1989; Kelland *et al*., 1995; Hagopian *et al*., 1999). Thus, the problem posed by cisplatin resistance appears to be more severe than has been acknowledged in the past. It

should be noted that although mechanisms of resistance have largely been derived from tissue culture studies, there is good evidence for a general agreement with mechanisms encountered clinically (Giaccone, 2000).

With the understanding that the cytotoxic effect of cisplatin is a complex process, extending from initial drug entry into cells to the final stages of apoptosis (see Figure 1), it follows that intracellular events interfering with any stage of this process will inhibit apoptosis and lead to drug resistance. Resistance mechanisms, therefore, arise as a consequence of intracellular changes that either prevent cisplatin from interacting with DNA, interfere with DNA damage signals from activating the apoptotic machinery, or both. Substantial evidence exists to indicate that the level and persistence of DNA adducts induced by cisplatin correlate directly with cytotoxicity (Fraval and Roberts, 1979; Roberts and Fraval, 1980). Reducing the extent of DNA damage, therefore, increases resistance, and this can occur through changes in drug accumulation, intracellular thiol levels, and/or DNA adduct repair. Thus, a reduction in the level of DNA adducts is generally ascribed to biochemical/molecular pharmacologic alterations, which are secondary to primary genetic changes. On the other hand, interference in initiating or transducing damage signals to inhibit apoptotic activation is due to changes at the molecular biologic/genetic level. Although a single mechanism of cisplatin resistance in a tumor cell is possible (Kelland *et al*., 1992b), in practice it is extremely rare. In general, resistance is multifactorial, in that several mechanisms are encountered simultaneously within the same tumor cell (Richon *et al*., 1987; Teicher *et al*., 1987; Eastman *et al*., 1988). Thus, the high level of resistance is a net effect of several unrelated mechanisms (Siddik *et al*., 1998), which compounds the difficulty in efforts to circumvent cisplatin resistance as a therapeutic strategy.

The specific mechanisms involved in cisplatin resistance are several, and discussed below in detail.

Reduced intracellular drug accumulation

There is ample evidence to indicate that reduced drug accumulation is a significant mechanism of cisplatin resistance. Reductions of the order of 20–70% have been documented in a variety of cell lines displaying resistance to cisplatin by a factor of 3–40-fold (Kelland, 1993). As expected from consideration of the multifactorial nature of the resistance mechanism, reduction in drug accumulation is not directly proportional to the level of resistance (Johnson *et al*., 1997). Indeed, the profile of resistance mechanisms of a given tumor cell line may not include defects in drug accumulation as a mechanism (Teicher *et al*., 1991; Kelland *et al*., 1992b). On the other hand, in some cancer cells, reduction in cisplatin accumulation is the principal mechanism of resistance, accounting for 70–90% of total resistance (Kelland, 1993).

The cause of the reduced cisplatin accumulation in resistant cells may be ascribed to either an inhibition in drug uptake, an increase in drug efflux, or both. A defect in the uptake process appears to be prevalent, but the mechanism for this remains obscure. Since reduced uptake can be demonstrated over a wide range of extracellular cisplatin concentrations, it is likely that resistance occurs as a result of changes in the nonsaturable process of passive drug diffusion (Yoshida *et al*., 1994; Kelland, 2000). There is limited evidence, however, that an energy-dependent active transport involving $Na^{+}K^{+}$-ATPase or a gated ion channel has a role in cisplatin uptake (Andrews *et al*., 1988; Gately and Howell, 1993), and, therefore, an alteration in this system as a causative factor in cisplatin resistance cannot be totally ruled out.

Development of resistance as a result of increased cisplatin efflux was largely discounted in earlier studies (Teicher *et al*., 1987; Andrews *et al*., 1988). More recently, there has been a resurgence of interest in this resistance mechanism as new exporter proteins have been identified. The multidrug resistance-associated (MRP) gene family, composed of at least seven members (MRP1–7), has been a major target of investigations, primarily as several of these ABC membrane proteins have been found in tumor cells and associated with cellular efflux of a variety of drugs (Borst *et al*., 2000). However, only MRP2 (cMOAT) appears to be important in cisplatin resistance, and this is consistent with the observation that resistant cells have increased levels of this transporter protein (Kool *et al*., 1997). Moreover, a 10-fold increase in resistance has been demonstrated in cells overexpressing *MRP2* following gene transfection (Cui *et al*., 1999). Support for the involvement of MRP2 in resistance also comes from the converse demonstration that transfection of tumor cells with an MRP2 antisense expression vector increases sensitivity to cisplatin (Koike *et al*., 1997). It is useful to note that MRP2 is not universally associated with cisplatin resistance (Shen *et al*., 2000). A second important area of investigation involving cisplatin efflux has centered around *ATP7A* and *ATP7B*, two copper-transporting P-type ATPase genes that are overexpressed in cisplatin-resistant tumor cells (Komatsu *et al*., 2000; Katano *et al*., 2002). More convincing has been the demonstration that human tumor cells transfected with *ATP7B* acquire significant resistance to both cisplatin (ninefold) and copper (twofold), primarily as a consequence of enhanced cisplatin efflux. The recent proposal to use overexpression of *ATP7B* as a clinical marker of chemoresistance to cisplatin in ovarian cancer affirms the potentially significant role of the copper transporter in cisplatin resistance (Nakayama *et al*., 2002).

Independent studies to involve either the multidrug resistance (MDR) P-glycoprotein pump (Smith *et al*., 1993; Wada *et al*., 1999; Bible *et al*., 2000) or the major vault/lung resistance-related protein (MVP/LRP) transporter directly (Mossink *et al*., 2002) in cisplatin efflux have been largely inconclusive. Caution, however, needs to be exercised since a clinical study in advanced ovarian cancer using a cisplatin-based treatment regimen has

demonstrated that P-glycoprotein overexpression is associated with a poor chemotherapeutic outcome (Baekelandt *et al*., 2000). Similarly, advanced ovarian cancers having increased levels of MVP/LRP respond poorly to cisplatin (Izquierdo *et al*., 1995). It is apparent that further studies are needed to clarify and/or amplify the roles of P-glycoprotein and MVP/LRP in cisplatin resistance.

Increased inactivation by thiol-containing molecules

The much lower chloride concentration ($\sim$4 mmol/l) in the cytoplasm facilitates aquation reactions, which activate cisplatin and enable it to react with, and become inactivated by a number of cytoplasmic constituents, including the abundant nucleophilic GSH and the cysteine-rich metallothionein. Concentrations of these thiol-containing molecules increase following chronic cisplatin exposure, and induce resistance by decreasing the level of the antitumor agent available for interaction with the target DNA. Inactivation of cisplatin by GSH and pathways promoting this reaction are shown in Figure 2.

Increases in GSH have been demonstrated in a number of cisplatin-resistant tumor models (Kelland, 1993), and confirmed in clinical studies (Wolf *et al*., 1987). Furthermore, in a panel of resistant ovarian tumor models, prominent elevations in GSH levels have been correlated directly with resistance. Such elevations may occur as a result of increased expression of the γ-glutamylcysteine synthetase (γ-GCS) gene (Mistry *et al*., 1991; Godwin *et al*., 1992; Hamaguchi *et al*., 1993), the translational product of which is a rate-limiting enzyme involved in GSH biosynthesis (Figure 2). These changes in GSH and γ-GCS appear to be mediated through upregulation of the transcription factor c-Jun (Pan *et al*., 2002). Resistance due to elevated GSH, however, is reversible and parallels the decline in this thiol molecule when cisplatin is removed from cell cultures (Hamaguchi *et al*., 1993). An increase in GSH following chronic cisplatin exposure, however, is not a general occurrence, and this likely contributes to the negative correlation in some studies between GSH levels and cisplatin sensitivity (D'Incalci *et al*., 1998; Kolfschoten *et al*., 2000).

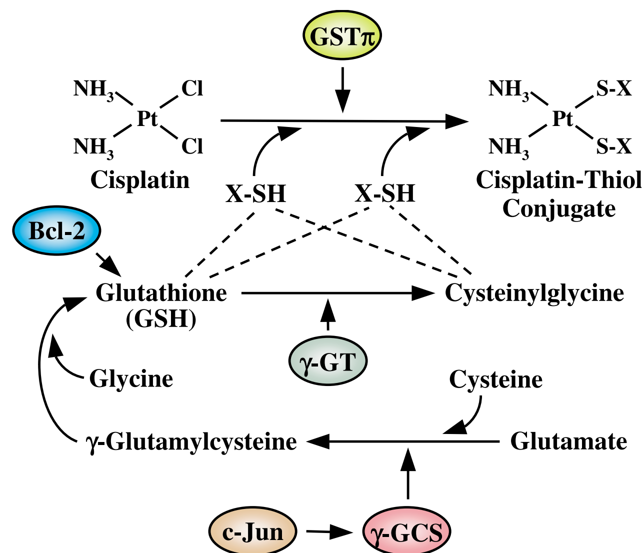

Figure 2 Inactivation of cisplatin by GSH. X-SH = glutathione or cysteinylglycine

The high reactivity of aquated cisplatin promotes its interaction with GSH in a nonenzymatic manner. This conjugation reaction, however, can also be catalysed by GSH-S-transferase π (GSTπ), which is a member of a family of enzymes involved in xenobiotic detoxication reactions (Goto *et al*., 1999). The increased expression of GSTπ (Sakamoto *et al*., 2001), together with elevated GSH levels in resistant tumor cells, suggests that enzymatic inactivation of cisplatin contributes significantly to the resistance phenotype at the clinical level. Indeed, a low level of GSTπ has been correlated to an overall survival rate of 82% with cisplatin in head and neck cancer patients, whereas a high level of the enzyme was associated with a twofold reduction in survival (Shiga *et al*., 1999). Overexpression of γ-glutamyltransferase (γ-GT) in cisplatin resistance is also observed, and this may further exacerbate inactivation of cisplatin (Daubeuf *et al*., 2002). γ-GT is a key player in GSH homeostasis, and generates cysteinylglycine during GSH catabolism (Figure 2). Since cysteinylglycine is 10-fold more reactive toward cisplatin than is GSH, the overproduction of the more reactive thiol by γ-GT is potentially a major contributor to GSH-mediated resistance.

Undoubtedly, the increased conjugation reaction between GSH and cisplatin is generally accepted as a significant factor in resistance, but other explanations for the effect of GSH are also of interest. These include the role of elevated GSH in either increasing DNA repair (Kelland, 1993) or increasing the inhibitory effect on apoptosis by buffering an endogenous drug-induced oxidative stress (Chiba *et al*., 1995; Slater *et al*., 1995). This is consistent with reports that cells overproducing the Bcl-2 protein have correspondingly higher intracellular GSH levels, which may contribute to the anti-apoptotic functions of Bcl-2 (Hockenbery *et al*., 1993; Chiao *et al*., 1995).

Metallothioneins are rich in thiol-containing cysteine molecules, which also provide ideal reactive centers for interaction with cisplatin, in much the same way as with GSH. It is not unexpected, therefore, that increases in metallothionein, up to fivefold over basal levels, have been observed in cisplatin-resistant murine and human tumor models (Kelley *et al*., 1988; Kasahara *et al*., 1991). It is noteworthy that in some studies, changes in metallothionein levels in resistant cell lines, or in human ovarian tumor biopsies taken before and after cisplatin-based therapy, have not been observed (Andrews *et al*., 1987; Schilder *et al*., 1990; Murphy *et al*., 1991). These variations in the reported data again emphasize the multifactorial nature of resistance and also that the increase in metallothionein is not necessarily an absolute requirement for cells to attain the resistance phenotype.

Increase in DNA damage repair

Formation and persistence of DNA adducts of cisplatin are vital in inducing apoptosis. Therefore, an enhanced rate of adduct repair will attenuate the apoptotic process. This is supported by the demonstration that an increased rate of repair is associated with an inhibition of drug-induced cytotoxicity in several murine and human tumor cell lines (Lai *et al*., 1988; Sheibani *et al*., 1989; Chao *et al*., 1991; Kelland *et al*., 1992a; Siddik *et al*., 1998). As with other mechanisms, repair is not universally present in all cisplatin-resistant cell lines (Schmidt and Chaney, 1993). When present, however, the contribution of increased repair to resistance is low, and usually results in resistance of the order of 1.5–2.0-fold. This limited increase is, nevertheless, considered as significant, and highlighted by the understanding that the inactivity of the transplatin congener is largely due to the rapid repair of its DNA adducts (Heiger-Bernays *et al*., 1990). The implied upper limit for repair capacity in resistance is supported by the finding that increased repair is unchanged even when resistance to cisplatin increases progressively in chronic drug exposure protocols (Chaney and Sancar, 1996; Eastman and Schulte, 1988). Moreover, topoisomerase II is linked to repair of cisplatin-induced DNA crosslinks, and it is not inconsistent to find that its overexpression in cases of clinical cancer is associated with the onset of cisplatin resistance (Ali-Osman *et al*., 1993; Hengstler *et al*., 1999). Factors contributing to enhanced repair are indicated in Figure 3.

NER is the major pathway for platinum adduct removal and repair of DNA damage. The significance of NER is highlighted by the finding that a cellular defect in this pathway results in hypersensitivity to cisplatin, and that restoration of NER integrity re-establishes sensitivity to normal levels (Chaney and Sancar, 1996; Furuta *et al*., 2002). NER has broad specificity, and no differences are observed in the excision of adducts induced by cisplatin and structurally diverse platinum-based drugs (Chaney and Vaisman, 1999). Indeed, enhanced repair of adducts in resistant cells also applies to platinum analogs that are effective against the resistance phenotype (Jennerwein *et al*., 1991), and this suggests that increased repair as a mechanism of resistance may be difficult to overcome through the platinum analog drug development process. Although the NER complex is composed of at least 17 different proteins (Sancar, 1994; Friedberg, 2001), it appears that upregulation of only a few rate-limiting proteins is necessary to increase the excision repair capacity in resistant tumor cells (Reed, 1998). For instance, cisplatin resistance is associated with increases in the excision repair crosscomplementing ERCC1 or ERCC1/XPF complexes, but not ERCC3 (Lee *et al*., 1993; Ferry *et al*., 2000). This finding with ERCC1 is of clinical relevance, as a twofold increase in *ERCC1* mRNA levels has been noted in patient's tumors that have become insensitive to cisplatin (Dabholkar *et al*., 1994). Similarly, the NER-related *XPA* gene is also overexpressed in cisplatin resistance and contributes to enhanced repair (Dabholkar *et al*., 1994). Conversely, testicular tumor cells, which are highly sensitive to cisplatin, express very low levels of XPA and ERCC1/XPF (Koberle *et al*., 1999).

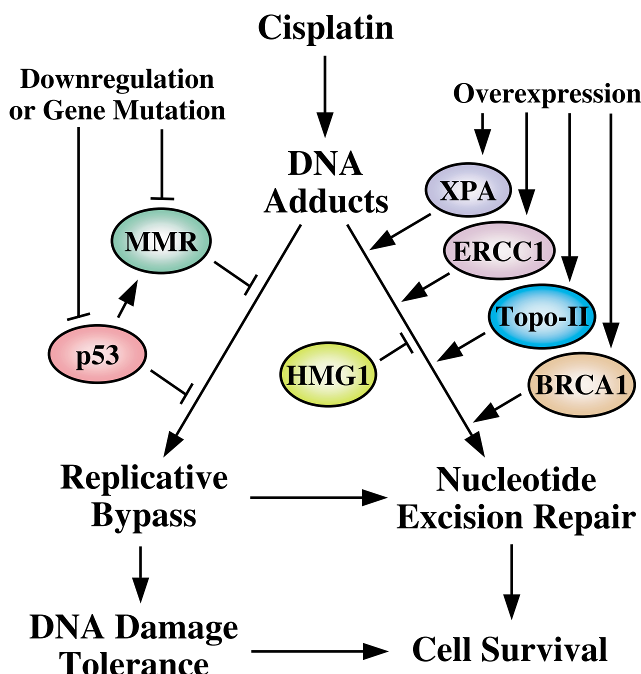

Figure 3 Factors modulating repair of cisplatin-induced DNA adducts and regulating replicative bypass

The NER complex is responsible for both global genomic and transcription-coupled nucleotide excision repair (TC-NER) of cisplatin-induced DNA adducts (Chaney and Sancar, 1996). An early signal for activation of the TC-NER pathway, which allows preferential repair of the transcribed strand of an active gene, is thought to be the stalling of RNA polymerase II at DNA helix-distorting lesions (Svejstrup, 2002). Several proteins, such as ERCC1 and XPA, play a key role in TC-NER, with ERCC1 demonstrating a preference for repairing interstrand platinum crosslinks in actively transcribed genes, such as the dihydrofolate reductase (*DHFR*) gene; ERCC1-mediated TC-NER of intrastrand lesions in *DHFR* gene is either inefficient or unchanged in resistant cells (Larminat and Bohr, 1994; Chaney and Sancar, 1996). Since intrastrand adducts are the critical cytotoxic lesions of cisplatin, and since assay techniques for gene-specific repair of interstrand crosslinks have been questioned, the significance of TC-NER in cisplatin resistance is considered by some as doubtful (Chaney and Sancar, 1996). This, however, is countered by the compelling demonstration that breast and ovarian cancer susceptibility gene *BRCA1* is involved in TC-NER (Gowen *et al*., 1998), and that overexpression or inhibition of this gene is associated with cisplatin resistance or sensitivity, respectively (Husain *et al*., 1998). Furthermore, cells deficient specifically in TC-NER are hypersensitive to cisplatin (Furuta *et al*., 2002).

Before repair is initiated, the damage to the DNA has to be recognized by specific proteins. Indeed, a number of DNA damage recognition proteins have been identified, but studies to define their involvement in cisplatin-resistant tumor cells have largely been confined to the MMR complex. It is noteworthy that MMR serves a critical role in maintaining the integrity of the genome through repair of DNA mismatch lesions, but it does not actually repair cisplatin adducts. A proposed viewpoint is that MMR attempts to repair the lesion, but in failing to do so activates the apoptotic signal (Vaisman *et al*., 1998). The MMR complex consists of a number of proteins, including hMSH2, hMSH6, hMLH1, hMutLα (heterodimer of hMLH1 and PMS2), and hMutSα (a heterodimer of hMSH2 and hMSH6), with hMSH2 and hMutSα involved directly in recognizing GpG intrastrand adducts of cisplatin (Duckett *et al*., 1996; Mello *et al*., 1996; Fink *et al*., 1998; Vaisman *et al*., 1998; Zdraveski *et al*., 2002). It is not surprising, therefore, that downregulation or mutations in MMR genes *hMLH1* or *hMSH2* are observed consistently in cisplatin resistance (Aebi *et al*., 1996; Drummond *et al*., 1996; Fink *et al*., 1996; Brown *et al*., 1997; Vaisman *et al*., 1998). Interestingly, loss of MMR in cisplatin resistance is associated with microsatellite instability and reduced apoptosis (Anthoney *et al*., 1996; Mayer *et al*., 2002). From the viewpoint of relevance, the level of resistance induced by the loss in MMR is about 2–5-fold, which is clinically significant. In contrast to the deficiency of MMR in cisplatin resistance, the alternative recognition protein HMG1 is overexpressed in resistant tumor cells (Nagatani *et al*., 2001). HMG1 is reported to shield DNA adducts from repair and its overexpression has been associated with cisplatin sensitivity (He *et al*., 2000), so the significance of increased levels of HMG1 in cisplatin resistance is not presently known.

In order to ensure genomic stability, it is vital that repair of DNA occurs prior to DNA replication. However, resistance arises when cells enhance their capacity to replicate DNA past the adduct, and then initiate postreplication repair (Chaney and Sancar, 1996). This in essence increases the ability of tumor cells to tolerate high levels of DNA adducts induced by cisplatin (Figure 3). In this respect, it is significant that replicative bypass is increased 3–6-fold by defects in hMLH1 or hMSH6, which attaches further importance to the role of MMR in cisplatin resistance (Vaisman *et al*., 1998). However, increased replicative bypass may also occur independent of MMR (Mamenta *et al*., 1994). It is noteworthy that increased tolerance to DNA adducts is not only seen in MMR deficiency but can also occur following p53 malfunction (see below). Indeed, p53 dysfunction exacerbates cisplatin resistance in MMR-deficient tumor cells (Lin *et al*., 2000, 2001), and this is consistent with both a downregulation of *hMSH2* by mutant p53 protein and an enhanced replicative bypass (Scherer *et al*., 1996). Moreover, loss of the p53 function accompanies MMR deficiency in cell lines selected for cisplatin resistance (Anthoney *et al*., 1996). Disruptions in crosstalks, as exemplified here between p53 and MMR, are probably at the center of the highly resistant phenotype.

Overexpression of HER-2/neu and the PI3-K/Akt pathway

The *HER-2/neu* proto-oncogene encodes a transmembrane receptor tyrosine kinase of 185 kD (p185), which has extensive homology to the epidermal growth factor receptor (EGFR) (Bargmann *et al*., 1986; Yamamoto *et al*., 1986). A poor response of human cancers to cisplatin is associated with amplification and overexpression of *HER-2/neu*, found in about 20–30% of breast and ovarian cancer patients (Slamon *et al*., 1989; Hengstler *et al*., 1999). Cisplatin resistance is similarly observed in model systems following transfection of tumor cells with an *HER-2/neu* expression vector (Tsai *et al*., 1995). Conversely, suppression of p185 activity by the tyrosine kinase inhibitor emodin or an antibody to the HER-2/neu receptor potentiates cisplatin cytotoxicity, which may in fact be mediated by a reduction in cisplatin-DNA adduct repair (Pietras *et al*., 1994; Zhang and Hung, 1996). However, contradictory results have also been observed in a few cases, as exemplified by an increase in cisplatin potency following induction of p185 tyrosine phosphorylation activity (Arteaga *et al*., 1994).

Once the HER-2/neu receptor is activated, downstream signaling is propagated through either the SHC/GRB2/SOS pathway, which in turn activates the Ras/MAPK pathway (see below), or the PI3-K/Akt pathway (Hung and Lau, 1999). Basal activity of the PI3-K/Akt pathway facilitates the induction of p21$^{Waf1/Cip1}$ by cisplatin in a p53-dependent manner, but without necessarily modulating Bax expression (Mitsuuchi *et al*., 2000). In contrast, *HER-2/neu* overexpression enhances the activity of Akt, which associates with p21$^{Waf1/Cip1}$ and phosphorylates the latter at threonine-145, thereby ensuring cytoplasmic localization of the CDK inhibitor (Zhou *et al*., 2001). The resulting diminution in nuclear levels of p21$^{Waf1/Cip1}$ by HER-2/neu overexpression may then explain the attenuation of cisplatin-mediated antiproliferative effects (Figure 4). Thus, p21$^{Waf1/Cip1}$ function can be either promoted or attenuated by the PI3/Akt, depending on the strength of the upstream signal. In addition, Akt promotes the phosphorylation of the Mdm2 oncoprotein and its translocation into the nucleus, where Mdm2 downregulates the p53 tumor-suppressor protein to induce resistance (Mayo and Donner, 2002; Oren *et al*., 2002; Zhou and Hung, 2002). The major cause for the onset of cisplatin resistance by HER-2/neu, however, may also be due to inactivation of the pro-apoptotic protein Bad following its phosphorylation by Akt (Hayakawa *et al*., 2000). Phosphorylation of Bad by ERK MAPK at an alternative site similarly attenuates cisplatin cytotoxicity (Hayakawa *et al*., 2000), and this may be exacerbated by HER-2/neu overexpression. To add to the complexity, the antiapoptotic signal may occur as a result of Akt-mediated phosphorylation of procaspase 9, which is then inactivated (Cardone *et al*., 1998). Moreover, this antiapoptotic signaling to suppress cisplatin cytotoxicity

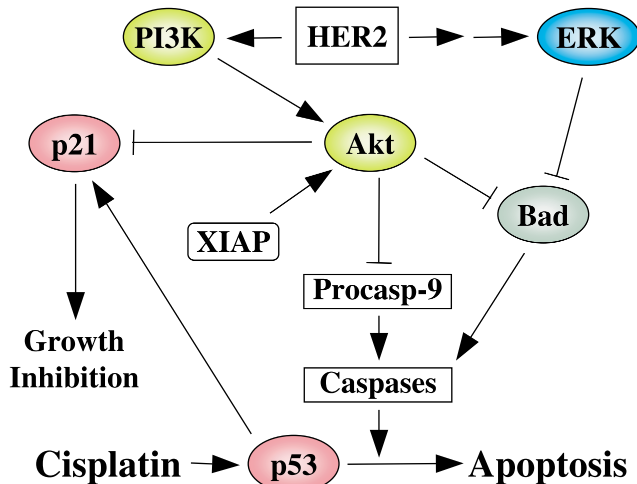

Figure 4 Cisplatin resistance affected through the HER-2/neu and PI3-K/Akt pathways

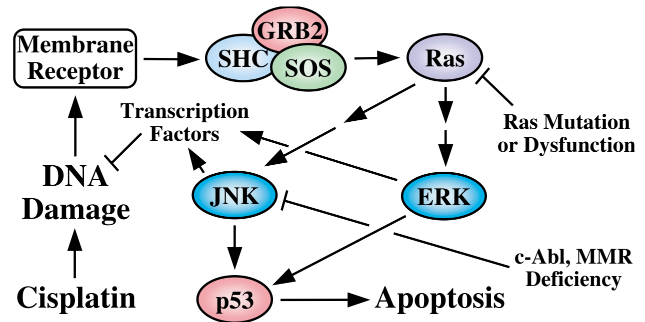

Figure 5 Attenuation of Ras and MAPK signaling pathways in cisplatin resistance

may include upregulation of Akt by XIAP (X-linked inhibitor of apoptosis protein) to facilitate inhibition of the caspase cascade (Asselin *et al*., 2001). How the PI3-K/Akt and MAPK signals are integrated downstream to induce either cell survival or cell death is not well understood. Evidence is apparent, however, for intricate crosstalk between several pathways, including those involving Akt, p53, and Mdm2, and the relative intensity and/or duration of each activated pathway may determine the final fate of cells (Gottlieb *et al*., 2002). Some of these pathways are depicted in Figure 4 (see also Figure 6).

Role of ras and MAPK pathway

As discussed earlier, MAPK subfamily members (p38, JNK, and ERK) are intimately associated with the mode of action of cisplatin. Whether a defect in the activation of MAPK pathway mediates cisplatin resistance is not clear, especially since some of the evidence points to both an increase and decrease in cisplatin sensitivity when the pathway is inhibited directly in human melanoma cells with PD98059, a specific MEK/ERK2 MAPK inhibitor (Mandic *et al*., 2001). Moreover, the increased sensitivity is seen in both cisplatin-sensitive and -resistant cell lines, drawing the rational conclusion that cisplatin resistance may not be related to the JNK1 or ERK1/2 MAPK pathway (Cui *et al*., 2000). Other studies, on the other hand, clearly establish the involvement of these pathways in mediating resistance, as is evident from studies utilizing the PD98059 inhibitor in a human cervical tumor cell system (Yeh *et al*., 2002). Furthermore, resistance appears following perturbation of the pathway by dysfunction of the H-Ras proto-oncogene, which is an upstream activator of JNK and ERK MAPK (Woessmann *et al*., 2002). This perturbation in the pathway is consistent with the finding that tumors expressing either *ras* mutation (Van't Veer *et al*., 1988) or *ras* overexpression (Fan J *et al*., 1997; Dempke *et al*., 2000) are resistant to cisplatin. It is also useful to note that activation of MAPK pathway by *ras* overexpression may not necessarily alter the tumor cell sensitivity to cisplatin (Holford *et al*., 1998). This inconsistency in the effect of *ras* overexpression on cisplatin resistance remains unexplained, as is the effect of the MEK/ERK2 MAPK inhibitor, but differences in the cellular context of the tumor models used in the reported studies are a good possibility.

When activated, the Ras/MAPK pathway contributes to post-translational modification of the tumor-suppressor/transcription activator p53 (Figure 5). In this regard, JNK MAPK activated via the MAP/ERK kinase kinase (MEKK1) phosphorylates p53, and a lack of this effect due to defective upstream activation of MEKK1 is the probable mechanism contributing to cisplatin resistance (Fuchs *et al*., 1998; Gebauer *et al*., 2000). The MAPK pathway also leads to the activation of a number of other transcription factors, such as c-Myc, c-Fos, and c-Jun (Robinson and Cobb, 1997; Martin-Blanco, 2000). These factors are overexpressed in cisplatin resistance, and their downregulation resensitizes tumor cells to the platinum drug (Kartalou and Essigmann, 2001; Pan *et al*., 2002). Since c-Fos and c-Jun are components of the AP1 transcription complex, which induces a number of genes, including ERCC1, metallothionein, and GST (Dempke *et al*., 2000), increased drug inactivation or DNA adduct repair will reduce DNA damage and provides a partial explanation for their effect in moderating cisplatin response. Similarly, c-Jun expression is closely linked to GSH levels (Pan *et al*., 2002), which inactivates cisplatin and further supports a reduction in DNA damage as a mechanism of cisplatin resistance mediated by overexpression of transcription factors (Figure 5). Interestingly, c-Fos and/or c-Jun is induced by cisplatin in both sensitive and resistant cells (Delmastro *et al*., 1997; Kartalou and Essigmann, 2001). These transcription factors, therefore, may act as both inhibitors and facilitators of apoptosis depending on the cell type and context (Leppa and Bohmann, 1999). Indeed, the levels of transcription factors are indirectly impacted by the functional status and effects of other molecular components on MAPK signaling. In this regard, it is noteworthy that JNK activity induced by cisplatin is substantially greater in tumor cells

demonstrating MMR proficiency than MMR deficiency (Nehme *et al*., 1997). Similarly, activation of c-Abl and p73 by cisplatin is necessary to facilitate apoptosis and is dependent not only on their wild-type gene status, but also on the cellular presence of hMLH1 and, therefore, the status of MMR (Nehme *et al*., 1997; Gong *et al*., 1999; Ono *et al*., 2001). From these considerations, it is not surprising that there is a link between c-Abl and JNK, and that cells lacking c-Abl become resistant to cisplatin by losing their ability to activate JNK (Kartalou and Essigmann, 2001).

Dysfunction of tumor-suppressor p53

Stabilization and activation of wild-type p53 are critical for cisplatin-mediated apoptosis. Therefore, tumor cells that have defects in the apoptotic function of p53 fail to activate the cell death program and enable them to become tolerant to DNA damage, which is a feature characteristic of resistance caused by disruption in signal transduction pathways (Kastan *et al*., 1991; Hartwell and Kastan, 1994; Pietenpol *et al*., 1994; Siddik *et al*., 1998, 1999). There is a significant body of evidence to indicate that tolerance to cisplatin adducts is of substantial significance in cisplatin resistance (Siddik *et al*., 1998). Indeed, an excellent correlation exists between DNA damage tolerance and the level of resistance (Johnson *et al*., 1997; Siddik *et al*., 1998; Yoshida *et al*., 1998). The ability to tolerate DNA adducts induced by the platinum agent is also seen clinically in a variety of tumor types, including those originating from the ovary and the head and neck (Marx *et al*., 1998; Righetti *et al*., 1999; Shiga *et al*., 1999; Cabelguenne *et al*., 2000).

A major factor affecting the loss of apoptotic function is *p53* gene mutation (see Figure 6), which is observed in about a half of all cancers (Hollstein *et al*., 1991; Soussi, 2000). Interestingly, there appears to be a correlation between *p53* gene status and cisplatin response among cancers considered sensitive to cisplatin; greatest response is observed in seminomatous germ cell tumors, which harbor predominantly wild-type *p53*, and a relatively lower response rate is noted in ovarian, head and neck, and metastatic bladder cancers, which demonstrate a 40–60% p53 mutation frequency (Sarkis *et al*., 1995; Houldsworth *et al*., 1998; Cabelguenne *et al*., 2000; Reles *et al*., 2001). When mutation does occur, it is commonly observed in exons 4–9 of p53, and this disrupts the ability of the tumor suppressor to bind to DNA and transactivate p53-dependent genes. The inability to transactivate bax specifically, and thereby prevent increase in the Bax : Bcl-2 ratio, is likely a major factor in affecting the resistant phenotype (Perego *et al*., 1996). It should be noted that many of the studies to define the impediment caused by mutant p53 have been conducted in tumor model systems. There is little doubt from several such studies that downregulation of the apoptotic process in tumor cells expressing mutant p53 is a major mechanism contributing to cisplatin resistance (Fan *et al*., 1994; Eliopoulos *et al*., 1995; Perego *et al*., 1996; Gallagher *et al*., 1997; Righetti *et al*., 1999). Since mutant p53 disrupts cell cycle arrest in G1, which is also the phase in which tumor cells are most sensitive to cisplatin, resistance due to loss in p53 function may be mediated in part by disruption in cell cycle checkpoints (Shah and Schwartz, 2001). Although such effects of mutant p53 abound, several contradictions have contributed to confusions regarding the role of mutant p53 in cisplatin resistance. For instance, the NCI panel of cell lines demonstrates a wide range of overlapping responses to cisplatin for the group of wild-type and mutant p53 tumor models, with some mutant p53 models expressing exquisite cisplatin sensitivity (O'Connor *et al*., 1997). These observations have also been documented in clinical cases, where tumors demonstrate either sensitivity or resistance to cisplatin irrespective of the *p53* gene status (Righetti *et al*., 1996). Other similar counterintuitive observations, with mutant p53 promoting sensitivity to cisplatin (Fan *et al*., 1995; Hawkins *et al*., 1996), suggest that the cellular context is an important variable in drug response. Moreover, the presence of mutation in *p53* may not necessarily negate wild-type p53 functions (Siddik *et al*., 1998). Since it is clear that the 5-year survival rate is significantly greater in patients with tumors expressing wild-type p53 than mutant p53 (van der Zee *et al*., 1995), the central role of wild-type p53 in facilitating cisplatin cytotoxicity cannot be ignored.

A significant understanding to emerge from collective consideration of the reported studies is that cisplatin resistance occurs irrespective of *p53* gene status. However, the resistance observed in cells harboring wild-type p53 can be substantially greater than that observed in tumor cells having mutant or null *p53* status (Siddik *et al*., 1998; Hagopian *et al*., 1999). This resistance in wild-type p53 cells is attributed to downregulation of cisplatin-mediated induction of wild-type p53 and its

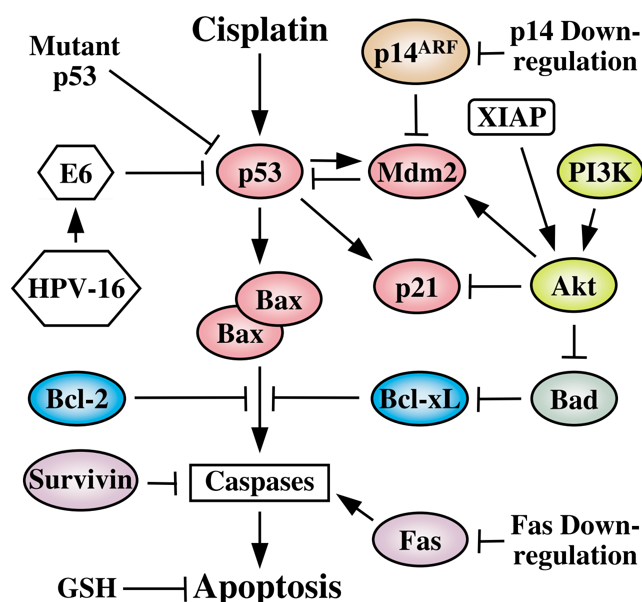

Figure 6 Disruption of p53-dependent apoptotic pathway in cisplatin-resistant tumor cells

inability to activate the apoptotic pathway (Figure 6). Intracellular factors that may inhibit such an activation of p53 include overexpression of the negative feedback regulator Mdm2 and downregulation of the moderator of Mdm2, p14^{ARF} (Fritsche *et al*., 1993; Shieh *et al*., 1997; Lakin and Jackson, 1999; Meek, 1999; Deng *et al*., 2002). However, investigations to define their role in cisplatin resistance are limited, and conclusions on the involvement of Mdm2 in resistance are conflicting (Kondo *et al*., 1995; Cocker *et al*., 2001). Nevertheless, recent evidence suggests that p53 function can indeed be attenuated by Mdm2 through a pathway involving *HER-2/neu* overexpression and resultant activation of the PI3-K/Akt pathway (Mayo and Donner, 2002; Oren *et al*., 2002; Zhou and Hung, 2002). The activity of wild-type p53 can also be attenuated by the human papillomavirus (HPV), which has been detected clinically in cancer of the cervix. In this case, the protein product of the *E6* oncogene in HPV-16 binds p53 to disrupt its transactivation and apoptotic functions, and causes platinum resistance (Kessis *et al*., 1993; Hagopian *et al*., 1999).

The apoptotic function of wild-type p53 is dependent on a number of cisplatin-induced upstream signaling pathways that stabilize and activate the tumor-suppressor protein by altering its phosphorylation and acetylation status (Fritsche *et al*., 1993; Shieh *et al*., 1997; Lakin and Jackson, 1999; Meek, 1999). It is not known, however, whether changes in these post-translational modifications of p53 affect resistance. The possibility that this may indeed occur is inferred from studies with a novel cisplatin analog that activates an independent DNA damage pathway to restore wild-type p53 function and, thereby, circumvent cisplatin resistance (Hagopian *et al*., 1999; Siddik *et al*., 1999).

Inhibitors of apoptosis

Molecular factors inducing cisplatin resistance do so by ultimately inhibiting apoptosis (see Figure 6). Apoptotic inhibitor molecules, such as survivin and XIAP, exacerbate resistance when overexpressed (Asselin *et al*., 2001; Ikeguchi *et al*., 2002). These inhibitors directly or indirectly impact the activities of caspases, which are the direct effectors of apoptosis, irrespective of the DNA damage pathway mediating the apototic signal. For cisplatin, caspases 3, 8, and 9 are critical, and their activation is attenuated in resistant cells (Henkels and Turchi, 1999; Blanc *et al*., 2000; Asselin *et al*., 2001; Ono *et al*., 2001). The inhibition of caspases 3 and 8 activation in these cells may be due in part to downregulation of the apoptotic signal as a result of a lack of *Fas* expression following cisplatin treatment (Qin and Ng, 2002).

Members of the Bcl-2 family are key players in regulating apoptosis (Farrow and Brown, 1996; Hanahan and Weinberg, 2000; Schuler and Green, 2001). They are localized in the mitochondria and have either pro- or antiapoptotic functions. The members form either homodimers or heterodimers, but only an excess level of homodimers can inhibit (e.g. Bcl-2/Bcl-2) or induce (e.g. Bax/Bax) apoptosis. The proapoptogenic Bax/Bax homodimer facilitates caspase activation through release of mitochondrial factors that include cytochrome *c* and Smac/DIABLO. This understanding is consistent with the requirement for p53-mediated transactivation of *bax* to affect cisplatin cytotoxicity (Eliopoulos *et al*., 1995). In keeping with this understanding, overexpression of *bcl-2* is associated with cisplatin resistance, and this is likely facilitated by an increase in GSH levels (Hockenbery *et al*., 1993; Chiao *et al*., 1995) and compounded by the presence of mutant p53 (Strasser *et al*., 1994; Herod *et al*., 1996; Miyake *et al*., 1999). Similarly, increased levels of the anti-apoptotic protein Bcl-xL are also observed in resistant tumor cells (Gebauer *et al*., 2000), possibly as a result of inhibition of the negative regulator Bad by the PI3-K/Akt pathway (Hayakawa *et al*., 2000). Paradoxical findings, which indicate that *bcl-2* overexpression is associated with either improved survival of ovarian cancer patients receiving cisplatin (Herod *et al*., 1996) or increased sensitivity of tumor cells to cisplatin (Beale *et al*., 2000), serve to demonstrate our present limited knowledge of the highly complex apoptotic process.

Conclusion

Recently, we have witnessed a rapid expansion in our knowledge regarding molecular factors that not only play an intricate role in cisplatin's mode of action but

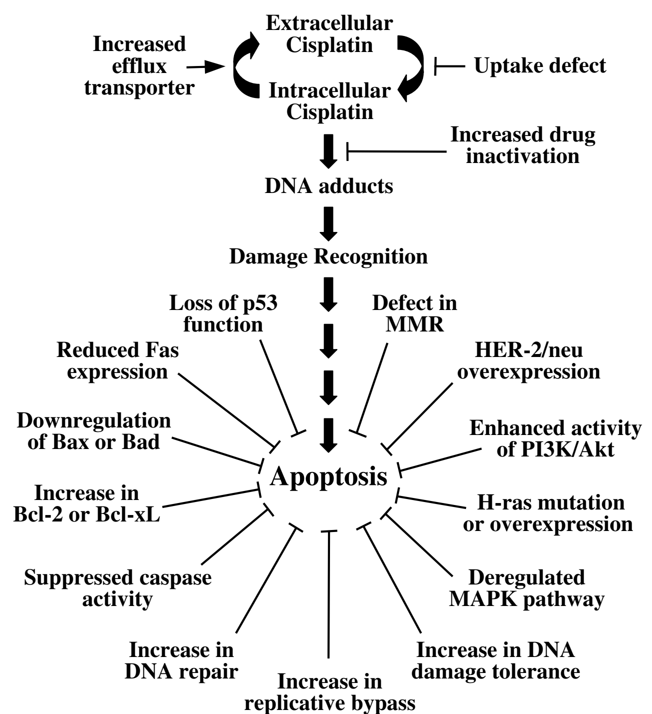

Figure 7 Mechanisms involved in inhibiting the apoptotic signal in cisplatin-resistant tumor cells. More than one mechanism is usually observed in resistant cells, and this contributes to the multifactorial nature of cisplatin resistance

also impede the ability of the drug to induce apoptosis. Downregulation of the apoptotic signal is essentially a universal characteristic of resistance, and some of the mechanisms associated with cisplatin resistance and discussed in the preceding sections are summarized in Figure 7. However, there are still major gaps that need to be filled in order to understand fully the delicate interplay between molecular factors that promote either death of the cancer cell or survival of the resistant phenotype. The additional knowledge is essential if we are to devise future strategies to circumvent multifactorial mechanism of cisplatin resistance more effectively and, more importantly, to translate them into durable clinical responses.

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