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Review paper

Cisplatin resistance and oncogenes—a review

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Cisplatin is among the most widely used broadly active cytotoxic anticancer drugs; however, its clinical efficacy is often limited by primary or the development of secondary resistance. Several mechanisms have been implicated in cisplatin resistance, including reduced drug uptake, increased cellular thiol/folate levels and increased DNA repair. More recently, additional pathways have been characterized indicating that altered expression of oncogenes that subsequently limit the formation of cisplatin–DNA adducts and activate anti-apoptotic pathways may also contribute to the resistance phenotype. Several lines of evidence suggest that expression of *ras* oncogenes can confer resistance to cisplatin by reducing drug uptake and increasing DNA repair; however, this is not a uniform finding. Tumor cells, in contrast to normal cells, respond to cisplatin exposure with transient gene expression to protect or repair their chromosomes. The c-*fos*/AP-1 complex, a master switch for turning on other genes in response to DNA-damaging agents, has been shown to play a major role in cisplatin resistance. In addition, AP-2 transcription factors, modulated by protein kinase A, are also implicated in cisplatin resistance by regulating genes encoding for DNA polymerase β and metallothionines. Furthermore, considerable evidence indicates that mutated p53 plays a significant role in the development of cisplatin resistance since several genes implicated in drug resistance and apoptosis (e.g. mismatch repair, *bcl*-2, high mobility group proteins, DNA polymerases α and β, PCNA, and insulin-like growth factor) are known to be regulated by the p53 oncoprotein. Improved understanding of molecular factors for the development of cisplatin resistance may allow the prediction of clinical response to cisplatin-based treatment. Furthermore, the identification of oncogenes involved in cisplatin resistance has already led to *in vitro* approaches which successfully inactivated these genes using ribozymes or antisense oligodeoxynucleotides, thus restoring cisplatin sensitivity. It is conceivable that these strategies, once transferred to a clinical setting, may have the potential to enhance the efficacy of cisplatin against a great variety of malignancies and thus more fully exploit the antineoplastic and curative potential of this drug. [© 2000 Lippincott Williams & Wilkins.]

Key words: Apoptosis, cisplatin resistance, gene therapy, oncogenes, resistance modifiers.

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Introduction

Cisplatin [*cis*-diamminedichloroplatinum (II), CDDP] is among the most widely used and broadly active cytotoxic anticancer drugs. However, the presence of primary or the emergence of secondary resistance significantly undermines the curative potential of cisplatin against many malignancies.[1] Considerable effort has been invested in defining cellular and molecular mechanisms responsible for cisplatin resistance. In recent years, a large number of potential determinants of cisplatin resistance have been identified in preclinical models including decreased drug accumulation, altered cellular thiol/reduced folate levels and increased repair of platinum–DNA damage (reviewed by Timmer-Bosscha *et al.*[2] and Chao[3]). While there have been reports of correlations between a number of these parameters and cisplatin-induced cytotoxicity, this has not been an invariable finding among the various human and murine tumor models studied so far.[3] The overall conclusion from published data therefore is that resistance in drug-selected cisplatin-resistant tumor cells is multifactorial and if there is a single critical molecular determinant it remains to be identified. In addition to these 'classical' resistance mechanisms, several additional pathways are currently being characterized indicating that cellular resistance to cisplatin may conceivably be based upon the overexpression or inactivation of certain oncogenes that consequently limit the formation of lethal platinum–DNA adducts, enabling the cell to tolerate platinum-induced DNA damage and activating anti-apoptotic pathways that counteract pro-

apoptotic processes initiated by cisplatin. Since data implicating altered oncogene expression as a mechanism of cisplatin resistance are gradually emerging,[3,4] the potential role of oncogenes in the development of cisplatin resistance will be reviewed.

Cisplatin resistance: the *ras*-mediated signal-transduction pathway

An increasing body of data suggests that *ras*-mediated signal-transduction pathways plays a major role in the expression of resistance to DNA-damaging agents. The *ras* supergene familiy (H-, K- and N-*ras*) is comprised of G proteins (molecular weight 21–29 kDa) that process the ability to bind guanine nucleotides.[5] These proteins function as molecular switches that control cell cycle, proliferation, cellular differentiation, cytoskeletal rearrangement, apoptosis, cellular defense mechanisms and nuclear import of proteins.[6] The *ras* proteins cycle between a GTP- and a guanosine diphosphate (GDP)-bound state. When bound to GDP, *ras* is incapable of activating signal-transduction pathways.[7] In its GTP-bound state, *ras* activates complex signal-transduction cascades including the MAP kinase kinase kinase (MAPKKK) Raf, the MAP kinase kinase (MAPKK) MEK and the extracellular signal-related

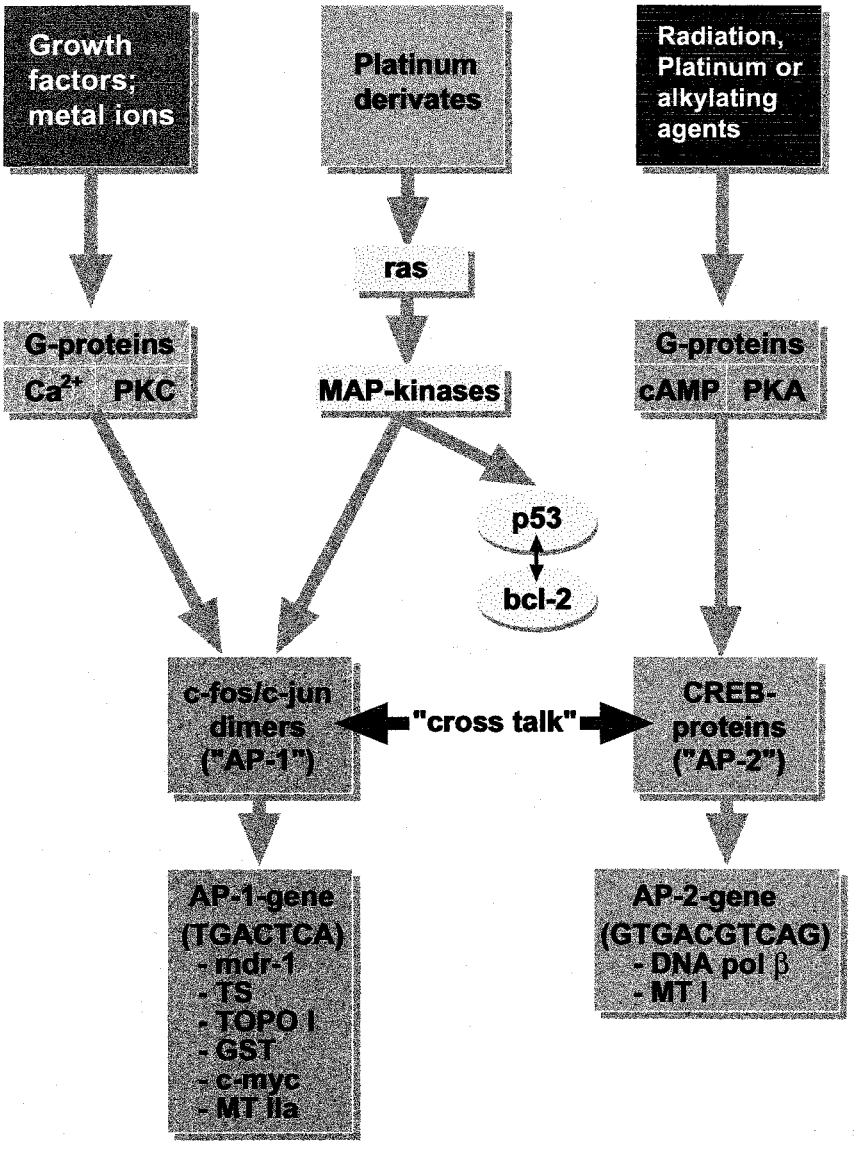

Figure 1. Model of the different signal-transduction pathways.

kinase (ERK) MAP kinase (MAPK).[8] This pathway ultimately leads to the activation of downstream transcription factors and proto-oncogenes such as c-*jun*, c-*fos* and c-*myc*, which in turn regulate expression of diverse proteins and play a critical role in cellular defense mechanisms.[6] *Ras* also activates the phosphoinositide 3-kinase pathway (PKC mediated) resulting in suppression of apoptosis (reviewed by Heimbrook and Oliff[9]). In addition, *ras*-regulated MAP kinases phosphorylate p53 suggesting that p53-mediated pathways are controlled by *ras*, thus *ras* genes may act as 'master genes' in the oncogene network implicated in cellular defense mechanisms.[10,11] A proposed model is outlined in Figure 1.

The first evidence that *ras* oncogenes may be involved in cisplatin resistance came from a study published by Sklar[12] who demonstrated that transfection of NIH 3T3 cells with *ras* oncogenes resulted in the expression of cisplatin resistance. Similar results have been described by Isonishi *et al.*[13] who showed that the observed cisplatin resistance was associated with decreased uptake of drug. To date these initial results have been confirmed by several other studies (Table 1). From these experiments it was concluded that *ras* oncogene transfection may result in reduced cisplatin uptake and enhanced DNA repair.[13–19] In contrast, other investigators provided evidence that *ras* gene expression may not be implicated in cisplatin resistance.[14,20] Holford *et al.*[21] examined a panel of 16 human ovarian carcinoma cell lines, and found no correlation between H-*ras* expression and cisplatin resistance. Similar results have been detailed by Kaufman *et al.*[22] who demonstrated that H-*ras* transfection of the human lung cancer cell line NCI-H82 did not alter cisplatin sensitivity. Using a human gall bladder carcinoma cell line, Masumoto *et al.*[23] failed to induce cisplatin resistance by H-*ras* transfection. However, in this study it was found that the *src* oncogene was able to induce cisplatin resistance. Before GTP-binding proteins such as *ras* can enter the GTPase cycle, they are transferred from the cytoplasm to the cell membrane since membrane localization is a critical parameter for *ras* function. The membrane localization is accomplished via a prenylation reaction, which involves attachment of a farnesyl group to the *ras* C-terminal cysteine (catalyzed by farnesyltransferase). Farnesyltransferase inhibitors have now been described,[24] suggesting that these compounds may interfere with *ras* activity and thus modulate the *ras*-controlled cisplatin resistance mechanisms. Support for this proposal came from a study published by Fokstuen *et al.*[25] who found that the farnesylation inhibitor BZA-5B increased cisplatin resistance in a human melanoma cell line, a finding that if confirmed may have clinical implications.

The precise role of *ras*-mediated signal transduction in cultured mammalian cells remains to be established. Published data are available which both support and refute the involvement of *ras* genes in the expression of cisplatin resistance. Since *ras* oncogene mutations are found in a wide variety of human cancers, additional studies are clearly needed to further eluciate the implication of the *ras*-regulated oncogene network on cisplatin resistance.

Cisplatin resistance: oncogenic transcription factors

DNA-damaging agents induce the expression of specific genes and such transcriptional modifications can influence cell cytotoxicity. Several lines of evidence suggest that cellular resistance to cisplatin is mediated by activation of so-called 'early-response genes' such as c-*myc*, c-*jun* and c-*fos*.[26] The proto-oncogene c-*fos* encodes a nuclear DNA binding

Table 1. Effects of a *ras* gene transfection on cisplatin resistance

Cell line	Origin	Oncogene	Cisplatin resistance	Reference
NIH 3T3	fibroblast	H-*ras*	increased	Isonishi *et al.*[13]
NIH 3T3	fibroblast	H-*ras*	increased	Niimi *et al.*[14]
NIH 3T3	fibroblast	H-*ras*	increased	Shinohara *et al.*[16]
NIH 3T3	fibroblast	K-*ras*	no change	Shinohara *et al.*[16]
NIH 3T3	fibroblast	*ras* (H, N, K)	increased	Sklar[12]
MCF-7	breast	H-*ras*	increased	Fan *et al.*[18]
SHOK	ovary	*ras* (H, N, K)	increased	Kinashi *et al.*[17]
HBL100	breast	H-*ras*	increased	Levy *et al.*[15]
Pam	keratinocytes	H-*ras*	increased	Perez *et al.*[19]
NCI-H82	lung	H-*ras*	no change	Kaufmann *et al.*[22]
HAG-1	gall bladder	H-*ras*	no change	Masumoto *et al.*[23]
A2780	ovary	H-*ras*	no change	Holford *et al.*[21]

phosphoprotein that, together with the product of the proto-oncogene c-*jun* or other members of the *jun* family (*junB* and *junD*), forms the heterodimeric (*fos/jun*) or homodimeric (*jun/jun*) transcription factor AP-1.[27] The members of this protein family share a conserved region, consisting of a leucine repeat dimerization domain (leucine zipper).[28] AP-1 is a collective name for a class of transcription factors that have been characterized by their ability to bind to the promoter/enhancer elements containing the TGACTCA sequence.[29] AP-1 proteins are modulated by protein kinase C (PKC) and Rubin *et al.*[30] demonstrated that inhibition of PKC repressed c-*jun* transcription. PKC has a crucial role in signal transduction for a variety of substrate proteins. Cells exposed to DNA-damaging agents were found to have an altered PKC activity resulting in enhanced phosphorylation of a number of proteins and a dramatic shift of gene expression.[31] PKC expression has also been implicated in apoptosis since ribozyme inhibition of PKC induced apoptosis in human glioma cells.[32] AP-1 plays a major role in the regulation of various genes habouring AP-1 sites in their promotor (e.g. *mdr-1*, c-*myc*, topoisomerase I, thymidylate synthase, metallothionine IIa, glutathion-S transferase),[33] and is involved in cell proliferation,[34] differentiation,[35] tumorigenesis[36] and possibly also apoptosis.[37] One of the mechanisms of post-translational regulation of AP-1 is based on oxidation/reduction mediated by redox-factor 1 (Ref-1), a protein identical to the DNA repair protein apurinic/apyrimidinic endonuclease (APE).[38] Other studies have shown that p53 is also subject to redox regulation by APE/Ref-1.[38,39] Recently, Kaina *et al.*[27] have provided compelling evidence that c-*fos*/AP-1 plays a decisive and general role in the cellular defense against genotoxic agents which require DNA replication to induce chromosomal instability, since AP-1 binding is stimulated upon exposure of cells to DNA-damaging agents. Results of studies investigating the role of the *fos/jun* complex in cisplatin-resistant cells have come from one main group of workers.[40–42] These authors provided the first evidence that cisplatin resistance is correlated with up-regulated c-*fos* expression in human tumor cell lines. Conversely, down-regulation of c-*fos* gene expression using a novel ribozyme technique restored cisplatin sensitivity.[43,44] Similar results have been reported by Pan *et al.*[45] who have demonstrated that cisplatin resistance in the human ovarian carcinoma cell line A2780DDP could be completely reversed by a c-*jun* antisense oligodeoxynucleotide. From these studies it was concluded that inactivation of the *fos* oncogene may be a primary target for gene therapy of cisplatin resistance. Taken together, the experimental findings suggest a novel role for the *fos/jun* complex, i.e. to trigger (via activation of other genes) the resumption of DNA replication and DNA repair following DNA damage. Since induction of *fos/jun* belongs to the earliest detectable nuclear responses of mammalian cells after exposure to mutagens, it appears to have a protective role by regulating DNA repair and replication when the genome is severely damaged,[46] and may therefore be a critical target for gene therapy approaches to circumvent cisplatin resistance.

Another class of regulatory elements that contributes to the transcriptional regulation by DNA-damaging agents are the cAMP responsive element binding (CREB) proteins (AP-2). This family of proteins has also been implicated in cAMP-, calcium- and viral-induced alterations of transcription.[47] AP-2 transcription factors are modulated by protein kinase A (PKA) and bind to promoter/enhancer elements containing the GTGACGTCAG sequence.[48] AP-2 plays a significant role in the regulation of the metallothionine I gene and the gene for DNA polymerase β (Figure 1).[43,44] Both genes have been implicated in cisplatin resistance.[2] Recently, Cvijic and co-workers[49,50] have demonstrated that a mutant PKA confers resistance to cisplatin in CHO cells suggesting that the cAMP-dependent signal-transduction pathway may play a major role in the development of cisplatin resistance. In addition, induction of the cAMP signal-transduction pathway also increases c-*fos* transcription.

The *myc* family proteins are comprised of several motifs that are commonly associated with transcription factors. The association of *myc* expression with tumor cells and proliferating normal cells led to the hypothesis that *myc* provides an important signal for cell growth and tumorigenesis (reviewed by Evan and Littlewood[51]). However, it is extremely difficult to define the genes that are directly regulated by *myc*, since it may send signals that trigger several additional cascades of gene expression through various pathways.[52] The expression of the c-*myc* gene is also regulated by several mechanisms including the *fos*/AP-1 complex and PKC.[53] Using 16 different cisplatin-resistant human carcinoma cell lines, Warenius *et al.*[54] have demonstrated that cisplatin resistance was correlated with a c-*myc*-dependent overexpression of cyclin D1. Other groups have shown that transfection of murine tumor cells with the c-*myc* gene resulted in the expression of cisplatin resistance.[13,55,56] In contrast, Mizushima *et al.*[57] demonstrated that cisplatin resistance in human lung cancer cell lines did not correlate with the degree of amplification of the N-*myc* gene suggesting that N-*myc* may not contribute to the observed cisplatin resistance. In pharmacokinetic studies, Kinashi *et al.*[17] clearly demonstrated that

cisplatin uptake was significantly reduced in cisplatin-resistant Syrian hamster cells after transfection with the c-*myc* or v-*mos* gene—a finding that, if confirmed, may be of relevance for the understanding of oncogene-modulated drug uptake by tumor cells.

Cisplatin resistance: cell cycle control and modulation of apoptosis

Considerable evidence indicates that cisplatin kills tumor cells by initiating apoptosis.[55–58] Internucleosomal DNA cleavage and ultrastructural changes characteristic of apoptosis have been observed following cisplatin exposure in L1210 cells and Chinese hamster ovary cell lines.[58,59] Whether apoptosis is a clinically significant mechanism of cancer cell death following treatment with cisplatin, however, remains to be established. The specific mechanisms that trigger apoptosis in response to cisplatin have not yet been clarified. With increasing insight into the molecular regulation of apoptosis researchers have more and more focused on the balance between pro- and anti-apoptotic factors as mediators of cisplatin sensitivity.[60,61] One critical regulator of apoptosis in response to anticancer drugs is p53. Cisplatin and other DNA-damaging agents induce stabilization and nuclear translocation of p53.[62,63] One of the downstream effectors of p53 is $p21^{waf\text{-}1/CIP\text{-}1}$, a cyclin-dependent kinase inhibitor that mediates cell cycle arrest.[64,65] Although best characterized as a cell cycle regulator, $p21^{waf\text{-}1/CIP\text{-}1}$ may also protect cells from apoptosis.[66,67] After cisplatin-induced DNA damage p53 transactivates the p21 gene resulting in p21 overexpression and subsequently in dephosphorylation of the retinoblastoma gene product Rb.[68] The Rb protein is preferentially localized in the nuclear matrix and binds to a family of cellular transcription factors (E2F1-5). Binding of Rb protein to E2F-1 inactivates the transcriptional activity of E2F-1.[69,70] E2F-1 is mainly involved in the control of genes known to be involved in DNA replication (e.g. DNA polymerase α, dihydrofolate reductase and thymidylate synthase).[68] In addition, $p21^{waf\text{-}1/CIP\text{-}1}$ inhibits the expression of proliferating cell nuclear antigen (PCNA), a protein essential for DNA polymerases δ and ε (involved in repair of cisplatin-induced DNA damage) resulting in a p53-dependent G_1 arrest of DNA damaged cells.[71] Potentially compelling evidence implicating p53 in cisplatin resistance was provided by Gallagher *et al.*[72] who used p53 genetic suppressor elements (GSE). GSE expression decreased p53 protein levels resulting in an 8-fold increase in resistance to cisplatin in A2780 ovarian carcinoma cells. Similar results have been reported by Kern *et al.*[73] using human melanoma cells. Additional evidence is provided by several other studies demonstrating a role of p53 gene mutations or disruption for p53 downstream signaling in cisplatin resistance *in vitro* and *in vivo*.[74–78] From these studies it was concluded that p53 mutations result in a loss of G_1/S checkpoint control and abrogate the ability of p53 to mediate apoptosis in response to DNA damage. Furthermore, these data add weight to the proposal that inactivation of the p53 DNA-binding domain can confer resistance to cisplatin.

p53 also directly affects expression of downstream genes that regulate sensitivity to apoptosis, activating transcription of *bax* (promotes apoptosis) and repressing transcription of *bcl-2* (inhibits apoptosis).[4,79] The *bax* promotor contains consensus binding sites for p53 and its activity is up-regulated by wild-type, but not mutant, p53.[79] In contrast, transcription of *bcl-2* is repressed by wild-type p53. Thus, wild-type p53 can produce reciprocal changes in *bax* and *bcl-2* transcription that favor apoptosis.[80] An additional anti-apoptotic factor of the *bcl-2* family is *bcl-*x_L. Using an *in vitro* system, Simonian *et al.*[81] demonstrated that overexpression of *bcl-*x_L provided an even better protection against cisplatin-induced apoptosis than *bcl-2*. Transfection of *bcl-2* or *bcl-*x_L conferred resistance and inhibited apoptosis following cisplatin exposure in several tumor models.[82–84] Likewise, Eliopoulos *et al.*[75] demonstrated a 3-fold increase in resistance to cisplatin by transfection of the *bcl-2* gene into A2780 human ovarian carcinoma cells.

Recently, Baserga *et al.*[85] have demonstrated that the insulin-like growth factor system (IGF-I/IGF-I receptor) can modulate apoptosis following exposure to DNA damaging agents. The mitogenic effect of IGF-I and its substantial importance for cell proliferation control and anti-apoptosis is well established.[86,87] The IGF system is involved in the regulation of main elements of the apoptotic cascade by controlling caspase-3, a key enzyme that initiates protein cleavage which subsequently activates DNA-cleaving enzymes.[88–90] Overexpression or stimulation of IGF-I-receptor inhibits caspase-3 activity either by stimulating *bcl-2* or by increasing phosphatidyl-inositol-3-kinase levels.[88–89] Recently, Ohlsson *et al.*[90] have demonstrated that transcription of the IGF-I receptor promotor is repressed by wild-type p53 and activated by the mutated protein, suggesting that mutated p53 may inhibit the caspase-3-mediated death-signaling pathways following exposure to DNA-damaging agents.[91] Support for this proposal came from two recently published studies.[92,93] They clearly showed that the expression of cisplatin resistance in HeLa cells

following fractionated X-irradiation was associated with increased (mutated?) p53 levels and reduced expression of interleukin-1β-converting enzyme (ICE)-related proteases (e.g. caspase-3). In addition, overexpression of the membrane receptor Apo1/Fas (CD 95) was found in these resistant cells.[93] From these studies it was concluded that p53-mediated reduced expression of ICE-related proteases may play a major role in the development of cisplatin resistance. It appears that the balance of pro- and anti-apoptotic factors, rather than a single parameter, predicts susceptibility towards apoptosis or cisplatin resistance. Further studies, however, are warranted to further eluciate how p53 mutations abrogate the apoptotic pathways in cisplatin-resistant cells.

Most recently, several studies have provided evidence that p53 is involved in DNA mismatch repair (MMR) and thereby in mechanisms associated with tolerance of DNA damage (review by Lage and Dietel[94]). In terms of cisplatin resistance, Anthoney *et al.*[95] demonstrated for the first time that loss of p53 function was accompanied by loss of MMR in the human ovarian carcinoma cell line A2880. Scherer *et al.*[96] provided the first evidence that MMR protein hMSH2 is down-regulated by mutated p53 protein resulting in enhanced bypassing mechanisms of DNA lesions. MMR deficiency was also associated with cisplatin resistance in other tumor models.[97–103] It is generally accepted that the MMR system interacts with the G_2 checkpoint, whereas the G_1 arrest is apparently independent of the MMR system. MMR-deficient cells, exhibiting resistance to cisplatin, showed a decrease in apoptosis, probably as a result of a decreased G_2 cell-cycle arrest.[99] It has been suggested that defects in the MMR system are linked to the pro-apoptotic factor *bax* and resistance to apoptosis.[104] Accordingly, Colella *et al.*[105] reported mutations in the *bax*-encoding gene as a consequence of microsatellite instability due to MMR deficiency. Recently, it has been postulated that cisplatin resistance associated with a deficient MMR system may result from the inability of the MMR-deficient tumor cells to detect DNA damage and to activate signal-transduction pathways leading to apoptosis and/or cell cycle arrest.[106] This hypothesis is supported by the observation that in tumor cells with a proficient MMR system cisplatin could activate c-Jun N-terminal kinase 1 (JNK 1) by a mechanism independent of p21-activated kinase 65 (PAKp65) more efficiently than in cells showing a reduced activity of the MMR system[106] while no activation could be observed in cells showing a deficiency in MMR. These observations support the assumption that the platinated-DNA-mediated activation of JNK 1 depends on the integrity of the MMR system. Furthermore, cisplatin activation of JNK 1 or other kinases by the MMR system could be part of a signal-transduction pathway that triggers apoptosis. Most recently, Jayaraman *et al.*[107] provided the first evidence that p53 can be activated by high mobility proteins (HMG). HMG proteins are a multifunctional family of small non-histone chromatin-associated proteins involved in gene regulation and maintenance of chromatin structure.[108] Several HMG family proteins specifically recognize cisplatin–DNA adducts, and binding to these adducts could trigger apoptosis and modulate cell cycle events. Using HeLa cell extracts, Jayaraman *et al.*[107] have shown that HMG-1 can increase p53 levels and is capable of bending DNA, suggesting that HMG-1 may activate p53 DNA binding following cisplatin damage by a novel mechanism involving a structural change in the target DNA.

To date, the precise role of p53 in modulating cisplatin resistance remains to be clarified since p53 acts as a transcription factor and controls the transcriptional activity of more than 300 genes partially implicated in cisplatin resistance mechanisms, such as DNA repair or repressing apoptosis pathways.[109] A hypothetical model of the p53-triggered cellular pathways is outlined in Figure 2.

Strategies for overcoming cisplatin resistance

The identification of oncogenes and tumor suppressor genes implicated as a fundamental mechanism of cisplatin resistance in human tumor cells has made it possible to consider these genes as potential targets to overcome drug resistance. An attractive approach to controlling gene expression and thus restoring cisplatin sensitivity is represented by antisense strategies using antisense oligodeoxynucleotides (ODN), on account of their target specificity and potential applicability to any sequenced gene. The mechanisms implicated in the action of antisense ODNs relate to RNase-mediated hydrolysis of the target mRNA/ODN hybrids or to translational arrest arising from steric hindrance by the RNA–DNA heteroduplex.[110] In models of human cisplatin-resistant tumor cells it has been shown that antisense ODNs targeting the oncogenes c-*myc*, c-*myb*, c-*jun* and *bcl-2* could indeed restore cisplatin sensitivity (Table 2), suggesting that this combination treatment might be a promising therapeutic approach in the clinical management of cisplatin-refractory patients. However, unsolved delivery/cellular uptake problems still limit their usefulness in cancer gene therapy.[111,112]

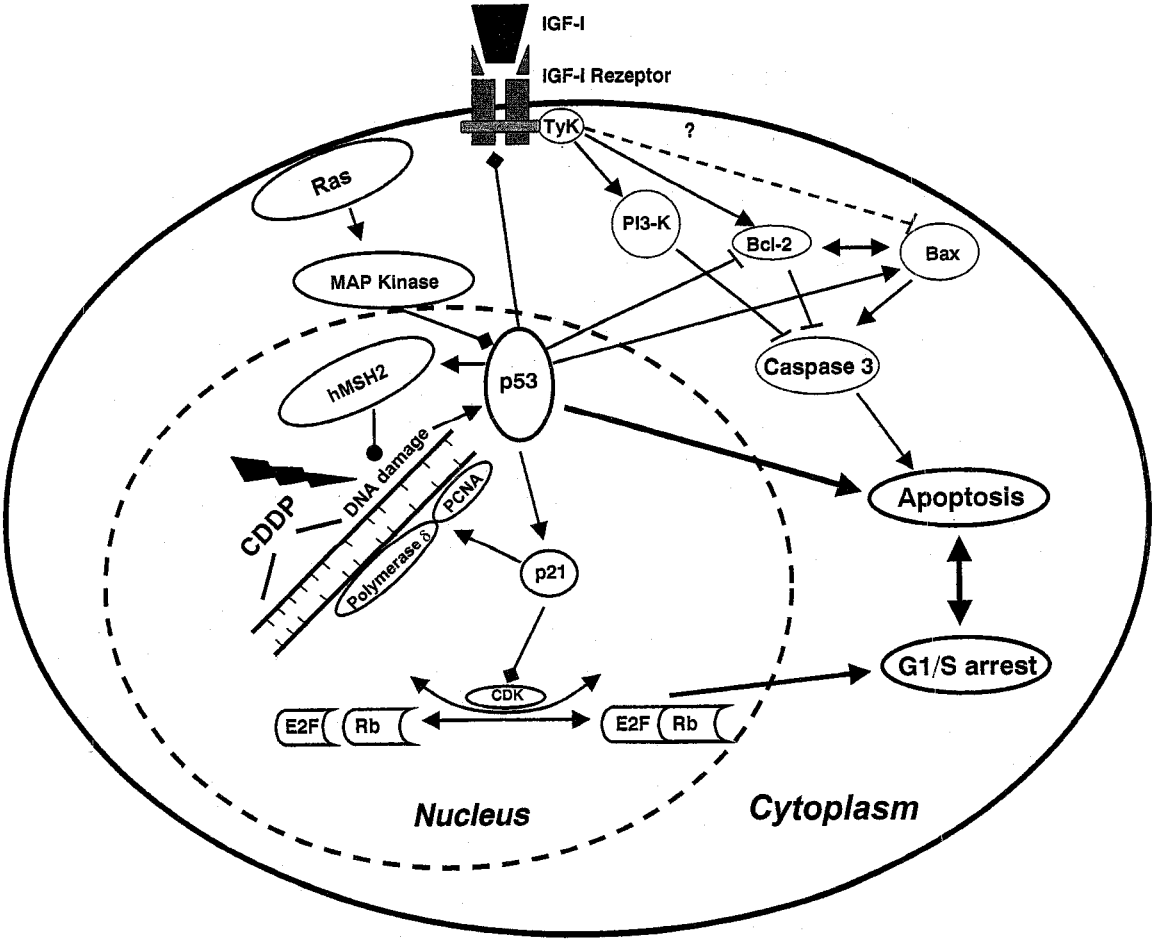

Figure 2. Model of the cellular interactions of p53.

Table 2. Preclinical gene therapy strategies to overcome cisplatin resistance *in vitro*

Target Gene	Cell line	Origin	Method	Cisplatin sensitivity	Reference
c-*fos*	A2780DDP	ovary	RZ	increased	Scanlon *et al.*[33]
c-*jun*	A2780DDP	ovary	ODN	increased	Pan *et al.*[45]
bcl-2	SW 2	lung	ODN	increased	Zangemeister-Wittke *et al.*[113]
c-*myc*	GLC4-cDDP	lung	ODN	increased	van Waardenburg *et al.*[56]
c-*myc*	T24/CDDP	bladder	ODN	increased	Mizutani *et al.*[114]
H-*ras*	EJ	bladder	RZ	increased	Funato *et al.*[41,42]
c-*myc*	M14	melanoma	ODN	increased	Citro *et al.*[115]
c-*myb*	LoVo	colon	ODN	increased	Del Bufalo *et al.*[116]

RZ, Ribozymes; ODN, antisense oligodeoxynucleotides.

Another possible approach to overcoming cisplatin resistance is the use of ribozymes. Ribozymes are RNAs that have site-specific RNA cleavage or ligation activities that may have therapeutic applications in human diseases.[117] The ability of anti-oncogene ribozymes to down-regulate c-*fos* and H-*ras* resulting in enhanced cisplatin-induced cytotoxicity has been demonstrated by Scanlon and co-workers (Table 2). However, a similar dramatic effect on cisplatin resistance was not seen in all cell lines examined, since additional factors (e.g. mRNA half-life, nuclease activity, stability of the target protein) may influence the efficacy of ribozymes within a cell. However, ribozymes may act as specific molecular probes to

further elucidate the effect of a given oncogene on cisplatin resistance.

Recently, two phase I clinical trials in cisplatin-refractory ovarian cancer[118] and non-small cell lung cancer[119] have been reported. In both trials, a recombinant adenovirus vector expressing wild-type p53 was injected directly into the tumor followed by cisplatin-based chemotherapy and two of 17 patients responded to this treatment in both trials. It therefore remains to be seen whether this new technology may have a positive impact on the clinical drug-resistance problem.

To date, several studies have provided significant evidence that cisplatin resistance could be circumvented *in vitro* and *in vivo* by modulation of PKA and PKC[50] using the protein kinase inhibitors 12-*O*-tetradecanoylphorbol-13-acetate,[13,120] quercetin[121] and forskolin.[122] However, in some cell lines examined, PKC activation and, in others, PKC inhibition seemed to potentiate cisplatin-induced cytotoxicity. This contradiction might be due to the measurement of PKC activity in cell lysates or in intact cells, since opposite effects on PKC were found.[2] In addition, there are several PKC isotypes and their individual contributions remain to be defined. Furthermore, it is important to remember that many of the so-called 'protein kinase inhibitors' show only very weak specificity, hampering valid interpretation of published data. Recently, the newly developed PKC inhibitor 7-hydroxy staurosporine (UCN-01) was found to restore cisplatin sensitivity in several murine and human tumor cell lines.[71,123–125] Based on these promising preclinical results in terms of restoring cisplatin sensitivity, UCN-01 in combination with cisplatin is currently undergoing phase I clinical trials in Japan and the US.

An improved understanding of the role of oncogenes in the development of cisplatin resistance may facilitate the prediction of clinical response to cisplatin-based treatment. In addition, continued efforts to understand the cellular and molecular mechanisms of cisplatin resistance could also identify novel targets for pharmacological or molecular intervention. Such efforts have the potential to enhance the efficacy of cisplatin against many types of cancers and so might allow exploitation of the full curative potential of the drug.

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