0026-895X/01/6006-1153–1160$3.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 60:1153–1160, 2001

Vol. 60, No. 6
912/949400
Printed in U.S.A.

MINIREVIEW

Identification of Genes That Mediate Sensitivity to Cisplatin

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Received February 23, 2001; accepted September 19, 2001

This paper is available online at http://molpharm.aspetjournals.org

ABSTRACT

Cisplatin (cDDP) is effective against some human tumors, but many are intrinsically resistant and, even among initially sensitive tumors, acquired resistance develops commonly during treatment. It has not been possible to prove which biochemical mechanisms control sensitivity to cDDP. Gene knockout studies in yeast, *Dictyostelium discoideum*, and mammalian cells have begun to unambiguously identify genes whose products function to modulate the cytotoxicity of cDDP. This review summarizes information currently available about the function of these genes. This comprehensive compilation points to the involvement of regulatory pathways known to mediate apoptosis, cell cycle checkpoint activation, and transcriptional rescue as regulators of cDDP sensitivity. Elucidation of the molecular mechanisms that mediate cDDP resistance holds promise for the design of pharmacological strategies for preventing, overcoming, or reversing this form of drug resistance.

The propensity of tumor cells to become resistant to cDDP with repeated exposure accounts for therapeutic failure in many cancer patients and is easily demonstrated in tissue culture [reviewed in Andrews and Howell (1990)] and animal models (Andrews et al., 1990). Determination of cDDP sensitivity in vitro using tumor samples or cell lines obtained before and after treatment of patients with cDDP indicates that the level of acquired resistance that emerges in vivo is usually only modest, in the range of 1.5- to 3.0-fold (Andrews et al., 1990; Andrews and Howell, 1990).

Biochemical studies have not succeeded in identifying conclusively the basis of resistance in any type of cell selected with cDDP, but they have defined several mechanisms that can contribute to resistance. The effectiveness of cell killing is a function of how much drug gets into the cell, how much of this actually reacts with DNA, how tolerant the cell is of lesions in its DNA, and how effectively it removes these adducts from DNA [reviewed in Perez (1998)]. Impaired uptake of cDDP is the most consistent finding both in vivo and in vitro (Sharp et al., 1995; Perez, 1998; Shen et al., 2000).

The difficulty of using biochemical approaches to obtain proof of the mechanisms responsible for acquired cDDP resistance has frustrated many investigators in the field. However, the rapid development of techniques for molecularly engineering cells to disrupt the expression of single genes has provided an alternative strategy that is potentially much less ambiguous. Although this approach is still in its infancy, it has already yielded novel insights into previously unsuspected mechanisms that control cDDP sensitivity. This article reviews recent information derived specifically from studies in which resistance to this important chemotherapeutic agent was produced in yeast, *Dictyostelium discoideum*, and mammalian cells by the disruption of a single gene. Although the focus of this review is on genes likely to play a role in acquired cDDP resistance (Table 1), there is a high likelihood that these same genes account for intrinsic resistance as well. At this juncture, it is not apparent that a useful distinction between acquired and intrinsic resistance can be made at the molecular level. Figure 1 provides a schematic that outlines some of the possible interactions between pathways in which the genes discussed below are believed to participate.

This work was supported in part by Grant NI602/1 from the German Research Foundation and Grant CA78648 from the National Institutes of Health. This work was conducted in part by the Clayton Foundation for Research–California Division. Drs. Christen and Howell are Clayton Foundation investigators.

ABBREVIATIONS: cDDP, cisplatin [*cis*-diamminedichloroplatinum(II)]; MMR, DNA mismatch repair; CPD, cyclobutane pyrimidine dimer; PKA, protein kinase A; APC, anaphase-promoting complex; HOX, homeotic complex; AP, activator protein; PIP5K, phosphatidylinositol 4-phosphatase 5-kinase; S1P, sphingosine-1-phosphatase; PDE, phosphodiesterase.

Genes Whose Products Are Involved in the Recognition or Processing of cDDP DNA Adducts

DNA Mismatch Repair Proteins. Several genes have been identified that when disabled seem to either impair or enhance recognition of cDDP adducts. The best known of these are the DNA mismatch repair genes, and hMSH2 in combination with one of its heterodimer partners binds specifically to cDDP adducts. Intriguingly, testicular and ovarian tissues, which give rise to tumors that are among the most sensitive to cDDP, have been reported to overexpress hMSH2 (Mello et al., 1996). This DNA repair system appears to function as a detector of cDDP adducts (Mu et al., 1997; Li, 1999; Nehme et al., 1999; Wu et al., 1999). Defects in DNA mismatch repair resulting from mutation or methylation-mediated silencing of hMLH1, hMSH2, or hPMS2 produce low level resistance to cDDP that seems to be caused by a failure to recognize the adduct and propagate a signal to the apoptotic machinery. cDDP treatment enriches malignant populations for cells that have lost DNA mismatch repair both in vitro (Fink et al., 1998b) and in vivo (Samimi et al., 2000). This topic has been reviewed (Fink et al., 1998a) and thus only a couple of very recent observations will be noted here.

In addition to directly causing cDDP resistance, loss of MMR function indirectly promotes the emergence of resistance to other drugs during treatment with cDDP. cDDP is a mutagen in mammalian cells. The molecular basis for its mutagenicity is believed to be related to bypass replication across cDDP adducts by the eukaryotic DNA polymerase β, and/or members of the class containing polymerases γ, ζ, and η (Crul et al., 1997; Chaney and Vaisman, 1999; Vaisman et al., 1999). This results in occasional misincorporation of non-complimentary bases by these polymerases that, if left unrepaired, generates point or frameshift mutations. An important replication-associated function of the MMR system may be to either prevent such bypass replication or correct the mismatches that are formed (Lin et al., 1999).

TABLE 1
Genes in which disruption produces cDDP resistance

Organism/Species	Gene	Reference
D. discoideum	Sphingosine-1-phosphate lyase 1	Li et al., 2000
D. discoideum	Similar to human P2Y purine receptor 1	Li et al., 2000
D. discoideum	Similar to phosphatidylinositol-4-phosphate 5-kinase from *Arabidopsis thaliana*	Li et al., 2000
D. discoideum	Disruption 280 bp upstream of the gene which is 51% similar to the human CAAX prenyl protease gene	Li et al., 2000
D. discoideum	Unidentified gene in an AT-rich intergenic region	Li et al., 2000
D. discoideum	Golgi vesicular membrane-golvesin	Li et al., 2000
D. discoideum	cAMP-specific phosphodiesterase RegA similar to *Drosophila melanogaster*/dunce gene	Li et al., 2000
Hamster	Regulatory subunit (RI) of the cAMP-dependent protein kinase (PKA)	Liu et al., 1996
Chicken	Lyn tyrosine kinase	Yoshida et al., 2000a
Mouse	c-Jun transcription factor AP1	Sanchez-Perez and Perona, 1999
S. cerevisiae	cAMP-specific phosphodiesterase PDE2	Burger et al., 2000
S. cerevisiae	ZDS2	Burger et al., 2000
S. cerevisiae	IRX1 high mobility group protein that has been isolated in yeast specifically binds cDDP-DNA adducts	Brown et al., 1993
S. cerevisiae	PHR1 photolyase	Chu, 1994

Recent studies from this laboratory have demonstrated that loss of MMR results in hypersensitivity to the ability of cDDP to generate variants resistant to high concentrations of etoposide, topotecan, gemcitabine, and paclitaxel (Lin et al., 1999). The ability of cDDP to generate resistant clones, and the fact that loss of MMR exacerbates this effect, may help explain the limited benefit of second-line therapy with any of these drugs in tumors that are initially sensitive to cDDP treatment (Lin and Howell, 1999). These results also raise the question of whether pretreatment screening of tumor samples for MMR activity might permit more effective identification of patients who are unlikely to benefit from treatment. Pretreatment analysis of tumor MMR protein levels is now possible, as shown by Lage and Dietel (1999) and Samimi (2000).

In addition to members of the MMR protein family, other proteins are known to interact with cDDP-damaged DNA. Ixr1/Ord1 is a yeast high mobility group (HMG) protein that specifically binds cDDP-DNA adducts (McA'Nulty et al., 1996; Mello et al., 1996). It encodes a transcription factor involved in oxygen regulation (Lambert et al., 1994; McA'Nulty et al., 1996). Compared with parental strains, *ixr1* deletion mutants contain fewer platinum-DNA adducts and are 2-fold resistant to cDDP (Brown et al., 1993; McA'Nulty et al., 1996). These data suggest that loss of *IXR1* may facilitate recognition and subsequent repair of these adducts. But other studies in mammalian cells by Zamble et al. (Zamble and Lippard, 1995; Zamble et al., 1996) showed that proteins containing the HMG domain DNA-binding motif, in particular rat HMG1 and a murine testis-specific HMG-domain protein, specifically inhibit excision repair of the intrastrand 1,2-d(GpG) and -d(ApG) cross-links produced by cDDP. As opposed to the findings in yeast, these results suggest that HMG-domain proteins can block excision repair of the major cDDP-DNA. Primer extension assays performed in the presence of HMG1 show that DNA polymerases, the MMR system and other damage-recognition proteins can also impart specificity to replicative bypass of cDDP-DNA adducts (Chaney and Vaisman, 1999; Vaisman et al., 1999).

The data from studies of *IXR1/ORD1* and *PHR1* in yeast, and the MMR genes in mammalian cells provide a firm foundation for the concept that alterations in the ability of the cellular machinery to recognize cDDP adducts can control sensitivity to the cytotoxicity of this drug. What is not yet clear is the extent to which the clinical phenomenon of acquired resistance can be ascribed to a mechanism that involves altered adduct recognition (Fig. 2).

Photolyases That Repair of Cyclobutane Pyrimidine Dimers. As an alternative or additional pathway to NER, a wide variety of organisms, including bacteria, fungi, plants, invertebrates, and many vertebrates, can revert cyclobutane pyrimidine dimers (CDPs) using CPD-photolyase in the presence of photoreactivating blue light (of wavelength 350–450 nm), which restore the bases to their native form (Yasui et al., 1994; Sancar, 1996). Pyrimidine dimers are among the

most common lesions produced in DNA by UV light. The *PHR1* gene of *Saccharomyces cerevisiae* encodes a photolyase that specifically and exclusively repairs this type of damage. Comparison of the regulatory sequences of this gene with those found in other damage-inducible genes from yeast cells revealed a conserved sequence that is also present in the *RAD2* and *RNR2* genes, and that is required for damage inducibility of all three. These sequences may constitute elements of a damage-responsive regulon in *S. cerevisiae* (Sebastian et al., 1990).

A possible role for eukaryotic photolyase activity was suggested when Fox et al. (1994) demonstrated that photolyase also binds to DNA damaged by cDDP and nitrogen mustard (HN_2) and to DNA alkylated by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). Mutations in photolyase were associated with resistance of yeast cells to cDDP, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, 4-nitroquinoline oxide, and HN_2. Transformation of yeast photolyase mutants with the wild-type *PHR1* gene increased sensitivity to these agents. Thus, although the binding of photolyase to DNA damaged by UV radiation aids survival of the cell, binding to DNA damaged by other agents may interfere with cell survival, perhaps by making the lesions inaccessible to DNA repair systems (Fox et al., 1994) or by persistently signaling the presence of an adduct that the enzyme cannot process.

An alternative mechanism is suggested by the results of Ozer et al. (1995) who demonstrated that *Escherichia coli* DNA photolyase binds specifically to the cDDP 1,2-d(GpG) intrastrand cross-link and stimulates the removal of the lesion by *E. coli* excision nuclease in vitro rendering them more resistant to cDDP killing. Todo et al. (1996) reported that there are two types of photolyase, one specific for cyclobutane pyrimidine dimers (CPD photolyase) and another specific for pyrimidine (6–4) pyrimidone photoproducts (6–4-photolyase).

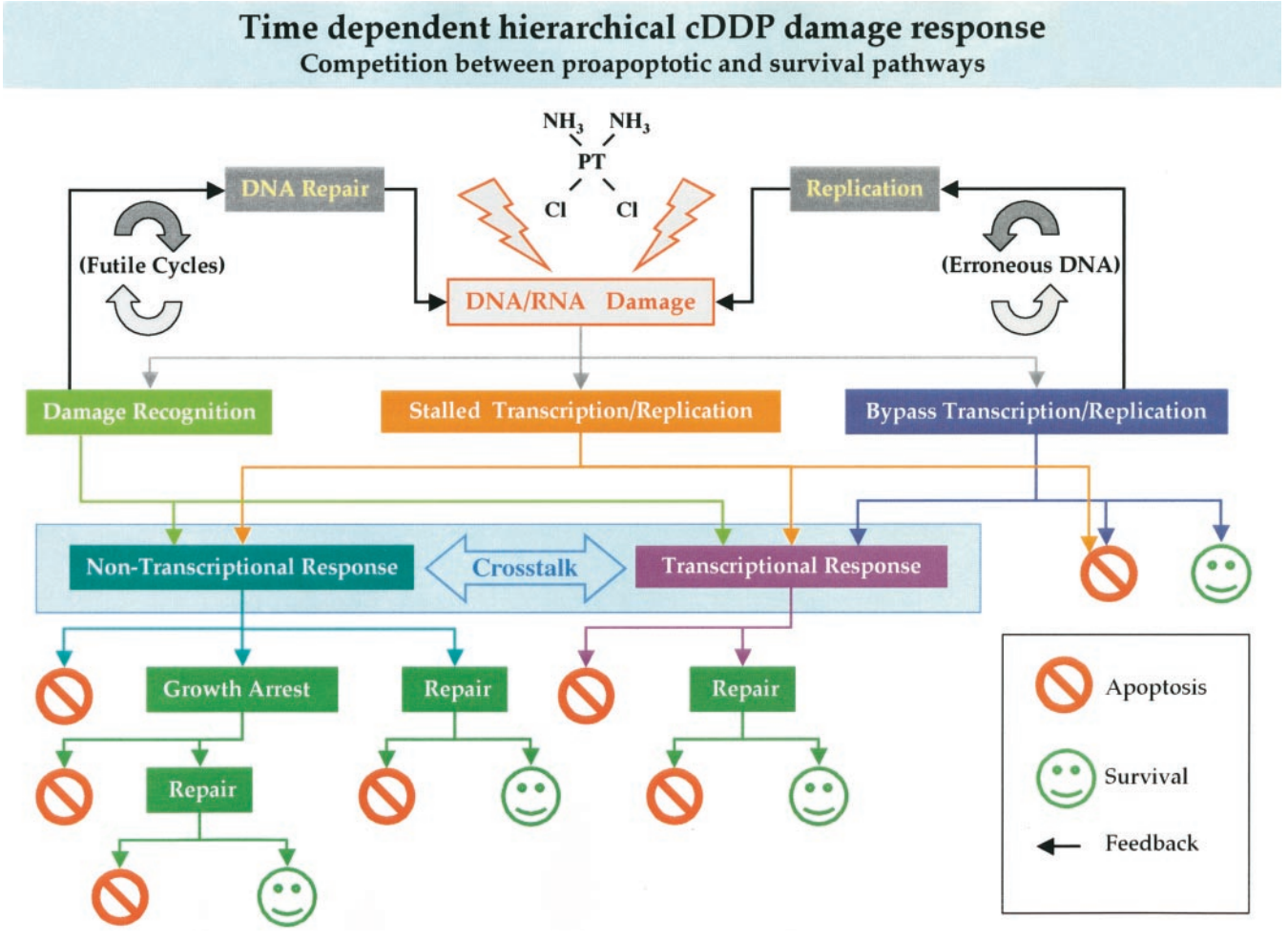

Fig. 1. DNA damage leads to simultaneous activation of proapoptotic and survival pathways in a time-dependent, hierarchical manner. Every transition in this response network (indicated by arrows) results from a perturbation of the steady-state levels of intracellular second messengers such as Ca^{2+}, cAMP, cGMP, sphingosine 1-phosphate/ceramide and inositol polyphosphates. Knockout of phosphodiesterase (RegA, PDE2), phosphatidylinositol-4-phosphate 5-kinase, or sphingosine-1-phosphate lyase 1 would be expected to reset the steady state and alter the damage response. Knockout of P2Y purine receptor 1, which is coupled to a G-protein, could also upset the second messenger balance. Knockout of genes such as the regulatory subunit (RI) of the cAMP-dependent protein kinase (PKA) is likely to alter the effect of changes in the concentration of second messengers. Knockout of the CAAX prenyl protease abrogates a specific part of the post-translational modification of important signal proteins (RAS, G-proteins, etc.) and could bias the damage response toward survival. Because the lyn tyrosine kinase is part of the mitogen-activated protein kinase pathway that activates the c-jun transcription factor, knockout of either of these two genes could potentially have a similar effect on cisplatin resistance. However, prediction of the effect of loss of function of a specific protein requires assessment of the effect on participating effectors at all different levels in the response network. In most cases, current evidence allows only evaluation of the net effect of the described knockout but does not permit finer resolution. The knockout of IRX1, ZDS2, and photolyase seems to play a role in DNA damage recognition, DNA repair, DNA accessibility, and cell-cycle regulation.

Chu et al. (1990) proposed that XPE, which is expressed at 5-fold higher levels in cDDP resistant tumor cell lines, may be the human homolog of CPD photolyase as this factor shares multiple binding characteristics with yeast CPD photolyase. More recently cryptochromes were proposed as possible human 6–4-photolyases, but no human homolog of the CPD photolyase has yet been cloned (Todo et al., 1996).

Genes of the Stress Signaling Pathways

Upstream factors involved in the cellular response to DNA damage mediate the induction of a network that transmits both pro- and antiapoptotic signals. Any interference that favors antiapoptotic signal transduction or abrogates proapoptotic pathways, including the transcriptional and translational response, is a potential mechanism of cDDP resistance. In view of the fact that most of these pathways also conduct signals generated by other intra- and extracellular stimuli, the importance of any given defect may be dependent on the other traffic on the pathways. One example is the abrogation of cDDP-induced apoptosis in human breast cancer cells by epidermal growth factor antisense RNA (Dixit et al., 1997).

P53 Pathway. It is well established that p53 plays a central role in chemotherapy-induced apoptosis; however, its importance as a determinant of cDDP sensitivity differs in various cell lines and tissues (Kastan et al., 1991; Kuerbitz et al., 1992; Fritsche et al., 1993; Eliopoulos et al., 1995; Juvekar et al., 2000). The strongest evidence that p53 normally functions to protect cells from cDDP-induced injury in hu-

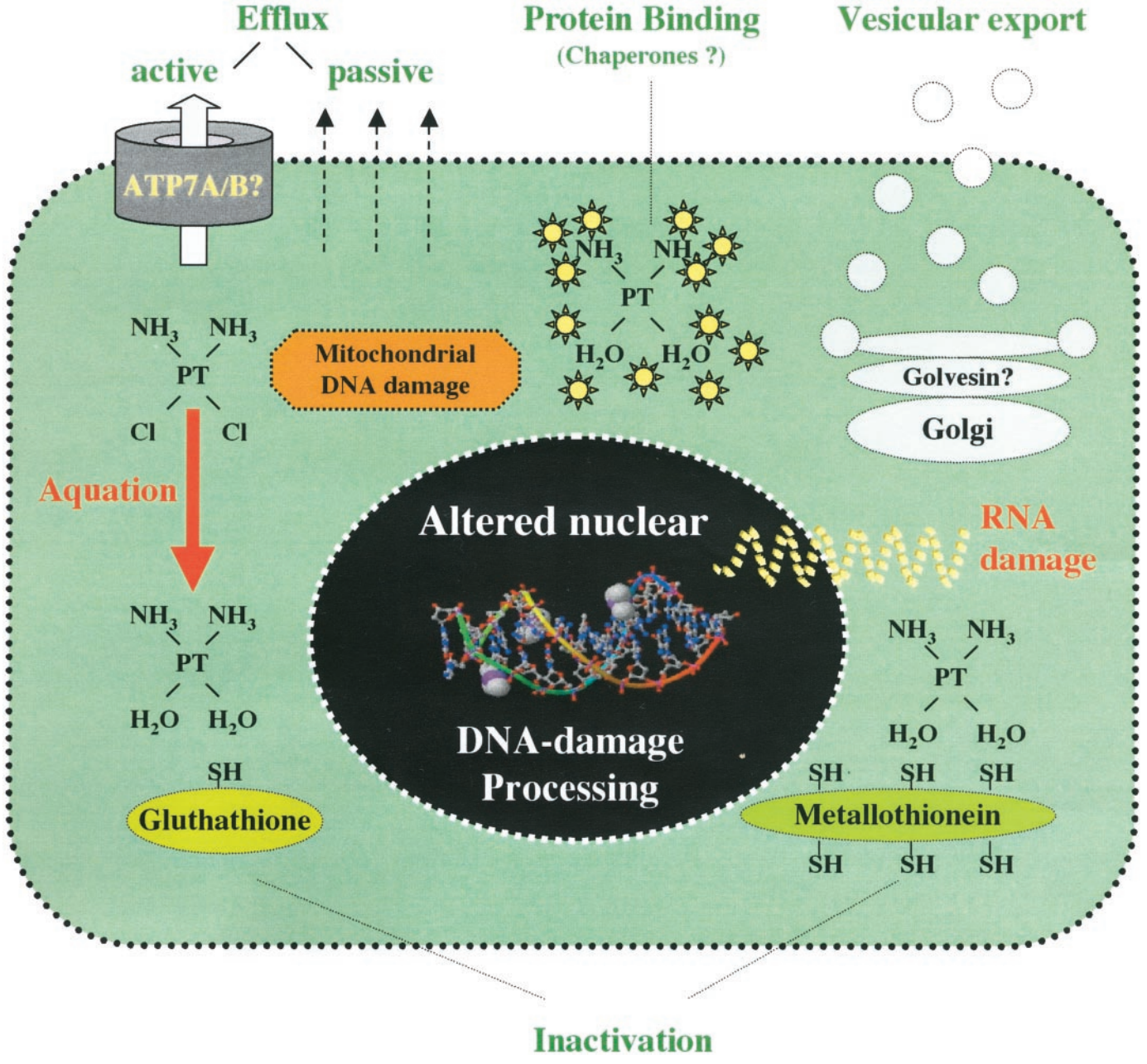

Fig. 2. Another general mechanism of cDDP resistance. Given the fact that cisplatin cytotoxicity results from the formation of DNA adducts, anything that decreases its cytoplasmic and hence nuclear concentration can be expected to render cisplatin less effective and the cell more resistant.

man tumor cells comes from studies of a subline of the HCT-116 human colon carcinoma in which both alleles of p53 were somatically disrupted using a knockout strategy (Bunz et al., 1999). In these cells, loss of p53 resulted in hypersensitivity to cDDP, possibly as a result of the failure to induce expression of the cyclin-dependent kinase inhibitor p21 (Bunz et al., 1999; Lin et al., 2000). The concept that loss of p53 function results in cDDP hypersensitivity is supported by studies in the A2780 human ovarian carcinoma cell line, in which lack of p53 function was found to be accompanied by cDDP hypersensitivity, loss of G_1/S checkpoint control, and decreased cDDP adduct repair compared with A2780 cells with intact p53 function (Pestell et al., 2000).

Eliopoulos et al. (1995) reported that Bcl-2 and p53 are frequently expressed in fresh biopsies of primary ovarian carcinoma and that either Bcl-2 or p53 or both are often overexpressed in resistant ovarian cancer cell variants. The authors attributed this to a progressive expansion of Bcl-2 and/or p53 positive subpopulations during the development of cDDP resistance (Eliopoulos et al., 1995), and suggested that Bcl-2 might act upstream of the p53 pathway. Expression of several antiapoptotic proteins has been directly linked to a change in cDDP sensitivity. Transfection of Bcl-2 or Bcl-XL has been shown to confer cDDP resistance and inhibit apoptosis after exposure to cDDP in several models (Miyashita and Reed, 1993; Dole et al., 1994; Fisher, 1994; Reed, 1994).

The elevated frequency of immunohistochemical staining for p53 observed in cDDP-pretreated tumors (Eliopoulos et al., 1995; Juvekar et al., 2000) implies that cDDP preferentially kills p53-deficient cells. However, this finding may also reflect overexpression of a partially or totally nonfunctional mutant protein because there is also evidence from other cell systems that p53 functions augment cDDP-induced apoptosis. Gallagher et al. (1997) isolated six independent genetic suppressor elements that target and disable p53 mRNA. When expressed in human ovarian carcinoma A2780 cells, they conferred up to 8-fold resistance to cDDP. The development of acquired resistance in A2780 cells has also been reported to be accompanied by loss of p53 function (Branch et al., 2000).

Dempke et al. (2000) described a more indirect role for p53 in the development of cDDP resistance through its regulation of 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).

In summary, the effect of the loss of p53 function on sensitivity to cDDP is not uniform in all cell types. It is likely that its influence depends on cell type-specific interactions with other pro- and anti-apoptotic pathways. This makes it unlikely that a measure of p53 protein level by itself will be a useful predictor of cDDP responsiveness. Given that the multiple interactions of p53 depend on several functional domains, a single mutation might well not abrogate all of them but rather shift the balance of downstream effects leading to either hypersensitivity or resistance.

The Protein Kinase A Pathway. *PDE2* codes for the *S. cerevisiae* cAMP phosphodiesterase [as opposed to mammalian nomenclature, which describes PDE2 as cGMP activated phosphodiesterases (Beavo, 1995)], which is a key regulator of intracellular cAMP level and is involved in the negative regulation of PKA activity (Sass et al., 1986; Burger et al., 2000). Interestingly, disruption of *PDE2* in yeast (Burger et al., 2000) and knockout of the RegA cAMP-phosphodiesterase in *D. discoideum* (Li et al., 2000) both produce cDDP resistance. Investigation of radiation-induced emergence of transient cDDP resistance in murine fibrosarcoma cells (SSK) has demonstrated involvement of cAMP- and cGMP-dependent signal transduction pathways in mammalian cells (Eichholtz-Wirth, 1995), although the effect was in the opposite direction in these human cells. The authors demonstrated a 1.8- to 2.5-fold increase in cDDP toxicity by increasing cAMP in both parental (nonirradiated) and resistant (irradiated) SSK cells.

Some information is available about how altered cAMP concentration may influence cDDP sensitivity. Based on studies with stable p53- and Ha-RAS-transformed human granulosa cells, Yoshida et al. (2000b) reported a synergistic effect of theophylline, a phosphodiesterase inhibitor, and cDDP with respect to induction of apoptosis. They attributed this phenomenon to the suppression of Bcl-2 expression. Increases in cAMP levels induced by forskolin, an adenylyl cyclase agonist, and 3-isobutyl 1-methylxanthine, a phosphodiesterase inhibitor, caused enhanced cDDP accumulation ($\approx$ 2 fold) in 2008 and A2780 human ovarian carcinoma cells relative to untreated cells. But Mann et al. (1991) found that the extent of the increased cDDP cytotoxicity was greater than the extent of cDDP accumulation and suggested that these drugs, or the increased cAMP levels they produce, not only modulate cDDP accumulation but also increase the cytotoxicity of the intracellular platinum. Although parental and resistant 2008 cells had identical cAMP-dependent protein kinase activity, the modulating effects of altering cAMP levels on cDDP accumulation and cytotoxicity were significantly diminished in DDP-resistant cells (Mann et al., 1991).

Yin et al. (2000) outlined evidence that the differential sensitivity to apoptosis and/or growth inhibition in the multidrug resistant phenotype of leukemic cells could be mediated via cAMP, partly through PKA via nuclear factor-κB and partly by PKA-independent pathways. However, it is important to note that the more resistant leukemia cell line (MDR1+ K/Dau600) had a higher expression of the PKA regulatory subunit RIα and nuclear catalytic subunit PKAcα, suggesting that these findings might be specific for tumor necrosis factor α-induced apoptosis and cytostasis.

Consistent with the concept that PKA activity maintains sensitivity to cDDP, wild-type Chinese hamster ovary cells transfected with and overexpressing the yeast phosphodiesterase, or a dominant negative mutant Rlα subunit, displayed increased resistance to cDDP (Liu et al., 1996; Cvijic and Chin, 1997). Furthermore, a 4- to 8-fold greater cDDP sensitivity was found in A2780 cells transfected with and overexpressing a functional R1α compared with their parental cells (Cvijic and Chin, 1998).

Chinese hamster ovary cells harboring a mutant R1α subunit selected for further resistance to cDDP remained refractory to cAMP-induced growth inhibition and had decreased PKA activity proportional to increasing levels of cDDP resistance (Cvijic and Chin, 1997). Gel shift analysis of the R1α-mutants showed increased binding of nuclear factor(s) to the damaged DNA (Liu et al., 1996; Cvijic and Chin, 1997). The PKA mutants also evidenced enhanced capacity for repair of DNA lesions as measured by a host cell reactivation of a

cDDP-damaged reporter plasmid (Liu et al., 1996; Cvijic and Chin, 1997). The authors suggested that functional inactivation of PKA may result in increased DNA repair and thereby facilitate resistance to DNA-damaging cytostatic drugs in cancer.

How any of the DNA repair mechanisms is linked to PKA remains uncertain, but one possible link was described by Heo et al. (1999). They suggested that *MCD1*/*SCC1*, which is involved in yeast sister chromatid exchange and DNA repair, is negatively regulated by the cAMP-dependent protein kinase A pathway via the anaphase-promoting complex (APC). APC is a large multiprotein complex required for the ubiquitination of mitotic cyclins and other regulatory proteins that are targeted for destruction during cell division (Page and Hieter, 1999). PKA signaling can also switch homeotic complex (HOX) transcription factors from transcriptional repressors to activators as shown for the HOX-PBX (pre-B-cell transformation-related gene product) complex (Saleh et al., 2000). Garcia et al. (1999) showed that PKA modulates AP-2 transcription factors, which regulate genes important for cDDP resistance (Dempke et al., 2000). In contrast to AP-1, AP-2 suppresses the expression of the heavy subunit of γ-glutamylcysteine synthetase. This enzyme is at least in part responsible for intracellular levels of glutathione in cDDP-resistant ovarian cancer cell lines (Yao et al., 1995). AP-2 has also been linked to increased human metallothionein IIa transcription, which is also found in some cells with acquired resistance to cDDP (Yang et al., 1998). It also remains possible that the regulative Rlα subunit regulates cDDP sensitivity via PKA independent pathways, especially in view of the identical PKA activity in cDDP-resistant and -sensitive 2008 ovarian cancer cells described by Mann et al. (1991).

Genes Whose Pathway Identification Is Not Yet Known

Investigators working with the slime mold *D. discoideum* have recently made an important contribution. Using an insertional mutagenesis technique, Li et al. (2000) identified 6 genes whose knockout produced cDDP resistance. Deletion of these genes did not result in resistance to other DNA-damaging agents, indicating an unusual degree of specificity for resistance to cDDP (Li et al., 2000). The cAMP-phosphodiesterase (RegA) has already been mentioned. The other 5 genes are golvesin, putative phosphatidylinositol 4-phosphatase 5-kinase (PIP5K), sphingosine-1-phosphatase (S1P), an unknown gene that is 52% similar to human purine receptor P2Y1, and an unknown gene that is 51% similar to the human CAAX prenyl protease.

Colocalization experiments by Molenaar et al. (2000) with a Golgi apparatus-selective stain and cDDP labeled with a fluorescent tag demonstrated that cDDP accumulated in vesicles associated with the Golgi apparatus. ATP7B, which serves to deliver copper from the cytoplasm into the *trans*-Golgi network, has been identified as a candidate for the transporter that mediates this sequestration on the basis of the observation by Komatsu et al. (2000) that overexpression of this protein rendered cells resistant to cDDP. This protein moves from the *trans*-Golgi network to the plasma membrane in response to exposure to copper (Petris et al., 1996). In this location, it exports copper from the cell, and conceivably might also export cDDP, if cDDP also triggers such relocalization. Golvesin is a Golgi-associated protein that putatively functions in vesicular membrane trafficking. Thus, if golvesin interferes with relocalization of cDDP export transporters from the Golgi to the plasma membrane, one might reasonably expect its deficiency to produce cDDP resistance.

PIP5K regulates the levels of several key signaling messengers, including phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate. It participates not only in signal transduction, cytoskeletal regulation, and DNA synthesis but also in vesicular trafficking (Yamamoto et al., 1995; Hassan et al., 1998). Although a human mutant PIP5K homolog has been implicated as possible cause for Friedreich's Ataxia (Carvajal et al., 1996), there is currently no discernible link to cDDP resistance in human or mammalian cell models.

S1P degrades sphingosine-1-phosphate and converts it into sphingosine. Impairment of S1P function shifts the balance between ceramide, which induces apoptosis via caspase activation, and sphingosine-1-phosphate, which promotes cell survival [for a review of sphingosine-1-phosphate function see Pyne and Pyne (2000)]. Although such a shift may reset the trigger for apoptosis, it is not currently apparent why this should be specific for cDDP-induced injury.

P2Y1 belongs to the metabotropic nucleotide receptor family, of which several subtypes (Communi et al., 2000) couple via G proteins to phospholipase C and the extracellular signal-regulated kinase/mitogen-activated protein kinase signal transduction pathways known to be involved in cellular proliferation and differentiation (Communi et al., 2000; Neary, 2000). In addition, P2Y receptors mediate inhibition of adenylyl cyclase and N-type Ca^{2+} channels and activation of K^{+} channels (North and Barnard, 1997). For this gene as well, it has not yet been possible to identify a link to biochemical mechanisms likely to influence cDDP sensitivity.

The final gene identified in the *D. discoideum* knockout experiments was CAAX prenyl protease, which is involved in the post-translational processing of proteins that contain a carboxyl-terminal CAAX motif via prenylation, endoproteolysis, and methylesterification (Ashby, 1998). One of these proteins is Ras, the most frequently mutationally activated oncogene found in human tumors (Petit et al., 1999). H-Ras and c-Fos were found to be over-expressed in the cDDP-resistant human colon carcinoma cell line (HCT8DDP) (Scanlon et al., 1989). Although activated Ras failed to induce cDDP resistance by itself, Masumoto et al. (1999) demonstrated that activated v-src can augment DNA repair leading indirectly to cDDP resistance. *V-SRC*-transfected HAG/src3–1 human gallbladder adenocarcinoma cells exhibited 3.5-fold resistance to cDDP compared with parental or mock-transfected HAG-1 cells [see also Dempke et al. (2000)].

Summary and Conclusions

Identification of the mechanisms that control sensitivity to cDDP is central to improving its therapeutic index, and to developing new agents that can prevent, overcome, or reverse the acquired resistance that emerges so commonly during treatment. This review discloses that many genes are already known whose disruption produces cDDP resistance of a magnitude that is likely to be of clinical significance. Because a tumor containing more than 10^{12} cells (≈1 kg) has a high

probability of containing cells harboring random mutations or deletions in many of these genes, it is likely that the application of the selective pressure of cDDP treatment enriches for clones whose resistance is mediated by a variety of different mechanisms. At this juncture, a skeptic would probably point out that even the gene knockout studies reviewed here have not yielded information that can be assembled into a comprehensive description of the biochemical pathways that mediate the cDDP-resistant phenotype. However, an optimist would likely counter that it is clearly possible to produce cDDP resistance by changing the expression of a single gene, and that this approach has already identified some of the most important individual steps in these pathways. Nevertheless, the challenge of sorting out which of these is central to the phenomenon of clinical cDDP resistance is very substantial.

This review also highlights the fact that the currently available information on cDDP resistance is derived from widely different model systems; in most of these, the biochemical and signaling pathways with the highest probability of contributing to cDDP resistance are poorly characterized. Given the powerful molecular tools now available, it seems that more rapid progress is likely to be made by focusing on thorough investigation of a single model system than by continuation of more limited studies in less-characterized cell types. In this regard, *S. cerevisiae* is a particularly good candidate model system, because many of the tools for doing this are currently in hand (Winzeler et al., 1999, 2000). The complete *S. cerevisiae* genome is now available and numerous cellular pathways, including key enzymes required for cell cycle and DNA repair are known. Taking into account the fact that, even in this organism, the knockout of just one gene may produce changes in hundreds of downstream elements involving many pathways, it will clearly be necessary to use new techniques to measure and interpret all of these changes. Emerging tools that permit simultaneous monitoring of the expression of several thousand genes and proteins using microarray technology, which are already being applied in the field of drug discovery (Friend, 2000; Scherf et al., 2000), should enable investigators to address this challenge. Although direct translation of functional information about a protein from *S. cerevisiae* to human cells is not always possible, once key pathways have been discovered, new drug targets can be identified that permit discovery of pharmacologic strategies for countering cDDP resistance (Friend, 2000).

The information reviewed here also forces one to consider whether current clinical trial strategies for investigating cDDP resistance need to be reconsidered. It is already apparent that this phenotype can be produced by many different mechanisms, some of which are quite specific for cDDP and others of which reduce sensitivity to disparate kinds of cellular stress. Particularly because even relatively low-evel resistance is sufficient to reduce tumor responsiveness to cDDP in vivo (Andrews et al., 1990; Nusbaum and Joseph, 1994; Fink et al., 1997), it seems likely that tumors that fail treatment with cDDP will contain clones that have adopted numerous different mechanisms with which to protect themselves against this drug. There is no practical way to assay for all of them in clinical samples using conventional methods. Thus, many clinical correlative studies currently planned or underway that focus on discovering associations between the expression of individual proteins and clinical resistance to cDDP seem unlikely to be a rich source of novel insights.

References

Andrews PA and Howell SB (1990) Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. *Cancer Cells* **2:**35–43.

Andrews PA, Jones JA, Varki NM, and Howell SB (1990) Rapid emergence of acquired *cis*-diamminedichloroplatinum(II) resistance in an in vivo model of human ovarian carcinoma. *Cancer Commun* **2:**93–100.

Ashby MN (1998) CaaX converting enzymes. *Curr Opin Lipidol* **9:**99–102.

Beavo JA (1995) Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. *Physiol Rev* **75:**725–748.

Branch P, Masson M, Aquilina G, Bignami M, and Karran P (2000) Spontaneous development of drug resistance: mismatch repair and p53 defects in resistance to cisplatin in human tumor cells. *Oncogene* **19:**3138–3145.

Brown SJ, Kellett PJ, and Lippard SJ (1993) Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin. *Science (Wash DC)* **261:**603–605.

Bunz F, Hwang PM, Torrance C, Waldman T, Zhang Y, Dillehay L, Williams J, Lengauer C, Kinzler KW, and Vogelstein B (1999) Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J Clin Invest* **104:**263–269.

Burger H, Capello A, Schenk PW, Stoter G, Brouwer J, and Nooter K (2000) A genome-wide screening in Saccharomyces cerevisiae for genes that confer resistance to the anticancer agent cisplatin. *Biochem Biophys Res Commun* **269:**767–774.

Carvajal JJ, Pook MA, dos Santos M, Doudney K, Hillermann R, Minogue S, Williamson R, Hsuan JJ and Chamberlain S (1996) The Friedreich's ataxia gene encodes a novel phosphatidylinositol-4-phosphate 5-kinase. *Nat Genet* **14:**157–162.

Chaney SG and Vaisman A (1999) Specificity of platinum-DNA adduct repair. *J Inorg Biochem* **77:**71–81.

Chu G (1994) Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. *J Biol Chem* **269:**787–790.

Chu G, Chang E, and Patterson M (1990) How cells recognize damaged DNA: clues from xeroderma pigmentosum and yeast. *Prog Clin Biol Res* **340A:**275–282.

Communi D, Janssens R, Suarez-Huerta N, Robaye B, and Boeynaems J (2000) Advances in signalling by extracellular nucleotides. the role and transduction mechanisms of P2Y receptors. *Cell Signal* **12:**351–360.

Crul M, Schellens JH, Beijnen JH, and Maliepaard M. (1997) Cisplatin resistance and DNA repair. *Cancer Treat Rev* **23:**341–366.

Cvijic ME and Chin KV (1997) Characterization of a cAMP-dependent protein kinase mutant resistant to cisplatin. *Int J Cancer* **72:**345–350.

Cvijic ME and Chin KV (1998) Effects of RIalpha overexpression on cisplatin sensitivity in human ovarian carcinoma cells. *Biochem Biophys Res Commun* **249:**723–727.

Dempke W, Voigt W, Grothey A, Hill BT, and Schmoll HJ (2000) Cisplatin resistance and oncogenes–a review. *Anticancer Drugs* **11:**225–236.

Dixit M, Yang JL, Poirier MC, Price JO, Andrews PA, and Arteaga CL (1997) Abrogation of cisplatin-induced programmed cell death in human breast cancer cells by epidermal growth factor antisense RNA. *J Natl Cancer Inst* **89:**365–373.

Dole M, Nunez G, Merchant AK, Maybaum J, Rode CK, Bloch CA, and Castle VP (1994) Bcl-2 inhibits chemotherapy-induced apoptosis in neuroblastoma. *Cancer Res* **54:**3253–3259.

Eichholtz-Wirth H (1995) Reversal of radiation-induced cisplatin resistance in murine fibrosarcoma cells by selective modulation of the cyclic GMP-dependent transduction pathway. *Br J Cancer* **72:**287–292.

Eliopoulos AG, Kerr DJ, Herod J, Hodgkins L, Krajewski S, Reed JC, and Young LS (1995) The control of apoptosis and drug resistance in ovarian cancer: influence of p53 and Bcl-2. *Oncogene* **11:**1217–1228.

Fink D, Aebi S, and Howell SB (1998a) The role of DNA mismatch repair in drug resistance. *Clin Cancer Res* **4:**1–6.

Fink D, Nebel S, Norris PS, Aebi S, Kim HK, Haas M, and Howell SB (1998b) The effect of different chemotherapeutic agents on the enrichment of DNA mismatch repair-deficient tumour cells. *Br J Cancer* **77:**703–708.

Fink D, Zheng H, Nebel S, Norris PS, Aebi S, Lin TP, Nehme A, Christen RD, Haas M, MacLeod CL, and Howell SB (1997) In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. *Cancer Res* **57:**1841–1845.

Fisher DE (1994) Apoptosis in cancer therapy: crossing the threshold. *Cell* **78:**539–542.

Fox ME, Feldman BJ, and Chu G (1994) A novel role for DNA photolyase: binding to DNA damaged by drugs is associated with enhanced cytotoxicity in Saccharomyces cerevisiae. *Mol Cell Biol* **14:**8071–8077.

Friend SH (2000) Genomic approaches to drug discovery. *Adv Oncology* **12**:3–11.

Fritsche M, Haessler C, and Brandner G (1993) Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene* **8:**307–318.

Gallagher WM, Cairney M, Schott B, Roninson IB, and Brown R (1997) Identification of p53 genetic suppressor elements which confer resistance to cisplatin. *Oncogene* **14:**185–193.

Garcia MA, Campillos M, Marina A, Valdivieso F, and Vazquez J (1999) Transcription factor AP-2 activity is modulated by protein kinase A- mediated phosphorylation. *FEBS Lett* **444:**27–31.

Hassan BA, Prokopenko SN, Breuer S, Zhang B, Paululat A, and Bellen HJ (1998) skittles, a *Drosophila* phosphatidylinositol 4-phosphate 5-kinase, is required for cell viability, germline development and bristle morphology, but not for neurotransmitter release. *Genetics* **150:**1527–1537.

Heo SJ, Tatebayashi K, and Ikeda H (1999) The budding yeast cohesin gene SCC1/MCD1/RHC21 genetically interacts with PKA, CDK and APC. *Curr Genet* **36:**329–338.

Juvekar AS, Adwankar MK, and Tongaonkar HB (2000) Effect of cisplatin-based chemotherapy on emergence of cisplatin resistance, and its correlation with intracellular glutathione levels and accumulation of p53 protein in human ovarian cancer. *Cancer Biother Radiopharm* **15:**295–300.
Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, and Craig RW (1991) Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* **51:**6304–6311.
Komatsu M, Sumizawa T, Mutoh M, Chen ZS, Terada K, Furukawa T, Yang XL, Gao H, Miura N, Sugiyama T, and Akiyama S (2000) Copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with cisplatin resistance. *Cancer Res* **60:**1312–1316.
Kuerbitz SJ, Plunkett BS, Walsh WV, and Kastan MB (1992) Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc Natl Acad Sci USA* **89:**7491–7495.
Lage H and Dietel M (1999) Involvement of the DNA mismatch repair system in antineoplastic drug resistance. *J Cancer Res Clin Oncol* **125:**156–165.
Lambert JR, Bilanchone VW, and Cumsky MG (1994) The ORD1 gene encodes a transcription factor involved in oxygen regulation and is identical to IXR1, a gene that confers cisplatin sensitivity to Saccharomyces cerevisiae. *Proc Natl Acad Sci USA* **91:**7345–7349.
Li G, Alexander H, Schneider N, and Alexander S (2000) Molecular basis for resistance to the anticancer drug cisplatin in Dictyostelium. *Microbiology* **146:**2219–2227.
Li GM (1999) The role of mismatch repair in DNA damage-induced apoptosis. *Oncol Res* **11:**393–400.
Lin X and Howell SB (1999) Effect of loss of DNA mismatch repair on development of topotecan-, gemcitabine-, and paclitaxel-resistant variants after exposure to cisplatin. *Mol Pharmacol* **56:**390–395.
Lin X, Kim HK, and Howell SB (1999) The role of DNA mismatch repair in cisplatin mutagenicity. *J Inorg Biochem* **77:**89–93.
Lin X, Ramamurthi K, Mishima M, Kondo A, and Howell SB (2000) p53 interacts with the DNA mismatch repair system to modulate the cytotoxicity and mutagenicity of hydrogen peroxide. *Mol Pharmacol* **58:**1222–1229.
Liu B, Cvijic ME, Jetzt A, and Chin KV (1996) Cisplatin resistance and regulation of DNA repair in cAMP-dependent protein kinase mutants. *Cell Growth Differ* **7:**1105–1112.
Mann SC, Andrews PA, and Howell SB (1991) Modulation of cis-diamminedichloroplatinum(II) accumulation and sensitivity by forskolin and 3-isobutyl-1-methylxanthine in sensitive and resistant human ovarian carcinoma cells. *Int J Cancer* **48:**866–872.
Masumoto N,Nakano S, Fujishima H, Kohno K and Niho Y (1999) v-src induces cisplatin resistance by increasing the repair of cisplatin-DNA interstrand cross-links in human gallbladder adenocarcinoma cells. *Int J Cancer* **80:**731–737.
McA'Nulty MM, Whitehead JP, and Lippard SJ (1996) Binding of Ixr1, a yeast HMG-domain protein, to cisplatin-DNA adducts in vitro and in vivo. *Biochemistry* **35:**6089–6099.
Mello JA, Acharya S, Fishel R, and Essigmann JM (1996) The mismatch-repair protein hMSH2 binds selectively to DNA adducts of the anticancer drug cisplatin. *Chem Biol* **3:**579–589.
Miyashita T and Reed JC (1993) Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* **81:**151–157.
Molenaar C, Teuben JM, Heetebrij RJ, Tanke HJ, and Reedijk J (2000) New insights in the cellular processing of platinum antitumor compounds, using fluorophore-labeled platinum complexes and digital fluorescence microscopy. *J Biol Inorg Chem* **5:**655–665.
Mu D, Tursun M, Duckett DR, Drummond JT, Modrich P, and Sancar A (1997) Recognition and repair of compound DNA lesions (base damage and mismatch) by human mismatch repair and excision repair systems. *Mol Cell Biol* **17:**760–769.
Neary JT (2000) Trophic actions of extracellular ATP: gene expression profiling by DNA array analysis. *J Auton Nerv Syst* **81:**200–204.
Nehme A, Baskaran R, Nebel S, Fink D, Howell SB, Wang JY, and Christen RD (1999) Induction of JNK and c-Abl signalling by cisplatin and oxaliplatin in mismatch repair-proficient and -deficient cells. *Br J Cancer* **79:**1104–1110.
North RA and Barnard EA (1997) Nucleotide receptors. *Curr Opin Neurobiol* **7:**346–357.
Nusbaum NJ and Joseph PE (1994) Emergence of resistance to VP-16/cisplatin chemotherapy: study in a lymphoblast model system. *Anticancer Drugs* **5:**457–462.
Ozer Z, Reardon JT, Hsu DS, Malhotra K, and Sancar A (1995) The other function of DNA photolyase: stimulation of excision repair of chemical damage to DNA. *Biochemistry* **34:**15886–15889.
Page AM and Hieter P (1999) The anaphase-promoting complex: new subunits and regulators. *Annu Rev Biochem* **68:**583–609.
Perez RP (1998) Cellular and molecular determinants of cisplatin resistance. *Eur J Cancer* **34:**1535–1542.
Pestell KE, Hobbs SM, Titley JC, Kelland LR, and Walton MI (2000) Effect of p53 status on sensitivity to platinum complexes in a human ovarian cancer cell line. *Mol Pharmacol* **57:**503–511.
Petit T, Izbicka E, Lawrence RA, Bishop WR, Weitman S, and Von Hoff DD (1999) Activity of SCH 66336, a tricyclic farnesyltransferase inhibitor, against human tumor colony-forming units. *Ann Oncol* **10:**449–453.
Petris MJ, Mercer JF, Culvenor JG, Lockhart P, Gleeson PA, and Camakaris J (1996) Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: a novel mechanism of regulated trafficking. *EMBO (Eur Mol Biol Organ) J* **15:**6084–6095.
Pyne S and Pyne NJ (2000) Sphingosine 1-phosphate signalling in mammalian cells. *Biochem J* **349:**385–402.
Reed JC (1994) Bcl-2 and the regulation of programmed cell death. *J Cell Biol* **124:**1–6.
Saleh M, Rambaldi I, Yang XJ, and Featherstone MS (2000) Cell signaling switches HOX-PBX complexes from repressors to activators of transcription mediated by histone deacetylases and histone acetyltransferases. *Mol Cell Biol* **20:**8623–8633.
Samimi G, Fink D, Varki NM, Husain A, Hoskins WJ, Alberts DS, and Howell SB (2000) Analysis of MLH1 and MSH2 expression in ovarian cancer before and after platinum drug-based chemotherapy. *Clin Cancer Res* **6:**1415–1421.
Sancar A (1996) No "End of History" for photolyases. *Science (Wash DC)* **272:**48–49.
Sanchez-Perez I and Perona R (1999) Lack of c-Jun activity increases survival to cisplatin. *FEBS Lett* **453:**151–158.
Sass P, Field J, Nikawa J, Toda T, and Wigler M (1986) Cloning and characterization of the high-affinity cAMP phosphodiesterase of Saccharomyces cerevisiae. *Proc Natl Acad Sci USA* **83:**9303–9307.
Scanlon KJ, Kashani-Sabet M, and Sowers LC (1989) Overexpression of DNA replication and repair enzymes in cisplatin- resistant human colon carcinoma HCT8 cells and circumvention by azidothymidine. *Cancer Commun* **1:**269–275.
Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, Kohn KW, Reinhold WC, Myers TG, Andrews DT, et al. (2000) A gene expression database for the molecular pharmacology of cancer. *Nat Genet* **24:**236–244.
Sebastian J, Kraus B, and Sancar GB (1990) Expression of the yeast PHR1 gene is induced by DNA-damaging agents. *Mol Cell Biol* **10:**4630–4637.
Sharp SY, Rogers PM, and Kelland LR (1995) Transport of cisplatin and bis-acetato-ammine-dichlorocyclohexylamine Platinum(IV) (JM216) in human ovarian carcinoma cell lines: identification of a plasma membrane protein associated with cisplatin resistance. *Clin Cancer Res* **1:**981–989.
Shen DW, Goldenberg S, Pastan I, and Gottesman MM (2000) Decreased accumulation of [14C]carboplatin in human cisplatin-resistant cells results from reduced energy-dependent uptake. *J Cell Physiol* **183:**108–116.
Todo T, Ryo H, Yamamoto K, Toh H, Inui T, Ayaki H, Nomura T, and Ikenaga M (1996) Similarity among the Drosophila (6–4)photolyase, a human photolyase homolog, and the DNA photolyase-blue-light photoreceptor family. *Science (Wash DC)* **272:**109–112.
Vaisman A, Lim SE, Patrick SM, Copeland WC, Hinkle DC, Turchi JJ, and Chaney SG (1999) Effect of DNA polymerases and high mobility group protein 1 on the carrier ligand specificity for translesion synthesis past platinum-DNA adducts. *Biochemistry* **38:**11026–11039.
Winzeler EA, Liang H, Shoemaker DD, and Davis RW (2000) Functional analysis of the yeast genome by precise deletion and parallel phenotypic characterization. *Novartis Found Symp* **229:**105–109.
Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, et al. (1999) Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. *Science (Wash DC)* **285:**901–906.
Wu J, Gu L, Wang H, Geacintov NE, and Li GM (1999) Mismatch repair processing of carcinogen-DNA adducts triggers apoptosis. *Mol Cell Biol* **19:**8292–8301.
Yamamoto A, DeWald DB, Boronenkov IV, Anderson RA, Emr SD, and Koshland D (1995) Novel PI(4)P 5-kinase homologue, Fab1p, essential for normal vacuole function and morphology in yeast. *Mol Biol Cell* **6:**525–539.
Yang YY, Robbins PD, and Lazo JS (1998) Differential transactivation of human metallothionein-IIa in cisplatin- resistant and -sensitive cells. *Oncol Res* **10:**85–98.
Yao KS, Godwin AK, Johnson SW, Ozols RF, O'Dwyer PJ, and Hamilton TC (1995) Evidence for altered regulation of gamma-glutamylcysteine synthetase gene expression among cisplatin-sensitive and cisplatin-resistant human ovarian cancer cell lines. *Cancer Res* **55:**4367–4374.
Yasui A, Eker AP, Yasuhira S, Yajima H, Kobayashi T, Takao M, and Oikawa A (1994) A new class of DNA photolyases present in various organisms including aplacental mammals. *EMBO (Eur Mol Biol Organ) J* **13:**6143–6151.
Yin Y, Allen PD, Jia L, MacEy MG, Kelsey SM, and Newland AC (2000) Constitutive levels of cAMP-dependent protein kinase activity determine sensitivity of human multidrug-resistant leukaemic cell lines to growth inhibition and apoptosis by forskolin and tumour necrosis factor alpha. *Br J Haematol* **108:**565–573.
Yoshida K, Weichselbaum R, Kharbanda S, and Kufe D (2000a) Role for lyn tyrosine kinase as a regulator of stress-activated protein kinase activity in response to DNA damage. *Mol Cell Biol* **20:**5370–5380.
Yoshida Y, Hosokawa K, Dantes A, Tajima K, Kotsuji F, and Amsterdam A (2000b) Theophylline and cisplatin synergize in down regulation of BCL-2 induction of apoptosis in human granulosa cells transformed by a mutated p53 (p53 val135) and Ha-ras oncogene. *Int J Oncol* **17:**227–235.
Zamble DB and Lippard SJ (1995) Cisplatin and DNA repair in cancer chemotherapy. *Trends Biochem Sci* **20:**435–439.
Zamble DB, Mu D, Reardon JT, Sancar A, and Lippard SJ (1996) Repair of cisplatin–DNA adducts by the mammalian excision nuclease. *Biochemistry* **35:**10004–10013.

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