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Review

Molecular mechanisms involved in cisplatin cytotoxicity

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Abstract. *cis*-diamminedichloroplatinum(II) or cisplatin is a DNA-damaging agent that is widely used in cancer chemotherapy. Cisplatin cross-links to DNA, forming intra- and interstrand adducts, which bend and unwind the duplex and attract high-mobility-group domain and other proteins. Presumably due to a shielding effect caused by these proteins, the cisplatin-modified DNA is poorly repaired. The resulting DNA damage triggers cell-cycle arrest and apoptosis. Although it is still debatable whether the clinical success of cisplatin relies primarily on its ability to trigger apoptosis, at least two distinct pathways have been proposed to contribute to cisplatin-induced apoptosis in vitro. One involves the tumour-suppressor protein p53, the other is mediated by the p53-related protein p73. Coupling cisplatin damage to apoptosis requires mismatch repair activity, and recent observations further suggest involvement of the homologous recombinatorial repair system. At present it is generally accepted that abortive attempts to repair the DNA lesions play a key role in the cytotoxicity of the drug, and loss of the mismatch repair activity is known to cause cisplatin resistance, a major problem in antineoplastic therapy. Clearly, a better understanding of the signalling networks involved in cisplatin toxicity should provide a rational basis for the development of new therapeutic strategies.

Key words. Cisplatin; anticancer drugs; DNA adducts; DNA repair; apoptosis; nucleolus.

Introduction

In 1965 Rosenberg and co-workers described the inhibition of *Escherichia coli* cell division by an electric current applied to the growth medium. Responsible for this effect was not the electric field in itself but the formation of tetravalent platinum complexes as the reaction product of platinum derived from the electrodes with ammonium chloride from the bacterial growth medium [1]. Subsequently, the study of diamminedichloroplatinum (II) compounds attracted much attention, and two isomers were identified, *cis*-diamminedichloroplatinum(II) (*cis*-DDP or cisplatin) and *trans*-diamminedichloroplatinum(II) (*trans*-DDP or transplatin). The compound active in inhibiting cell division was later shown to be cisplatin. In addition, it exhibited marked antitumour activity. Transplatin, in contrast, was poorly effective in both cases [2–4].

At present cisplatin is an important chemotherapeutic agent used widely in the treatment of many tumours, particularly testicular and ovarian [3, 5–7]. The related compounds carboplatin and oxaliplatin have also been used increasingly in recent years. This review aims to summarise and discuss current views on the cellular and molecular mechanisms of cisplatin action.

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Cisplatin makes DNA adducts

The reactivity of cisplatin is influenced by the surrounding chloride concentration. In blood and extracellular body fluids the physiological chloride concentration is about 100 mM, and cisplatin is relatively less reactive. Inside cells the chloride concentrations drop to a few millimolar, thus increasing cisplatin reactivity (see [8]). In aqueous solution, chloride ions in cisplatin are displaced to allow the formation of aquated species, which represent the reactive forms of the compound (fig. 1a). Nucleophilic groups containing oxygen, nitrogen or sulfur atoms with unpaired electrons can bind to platinum in substitution for chlorine ions. These groups are present in many amino acid side chains as well as the purine bases of DNA or RNA. However, the most relevant interactions are those involving DNA, and the formation of the monoaqua complex is likely to represent the rate-limiting step for cisplatin-DNA cross-linking. The aqua group is a good leaving group, and the positively charged complex is thought to be electrostatically attracted to the negatively charged DNA helix.

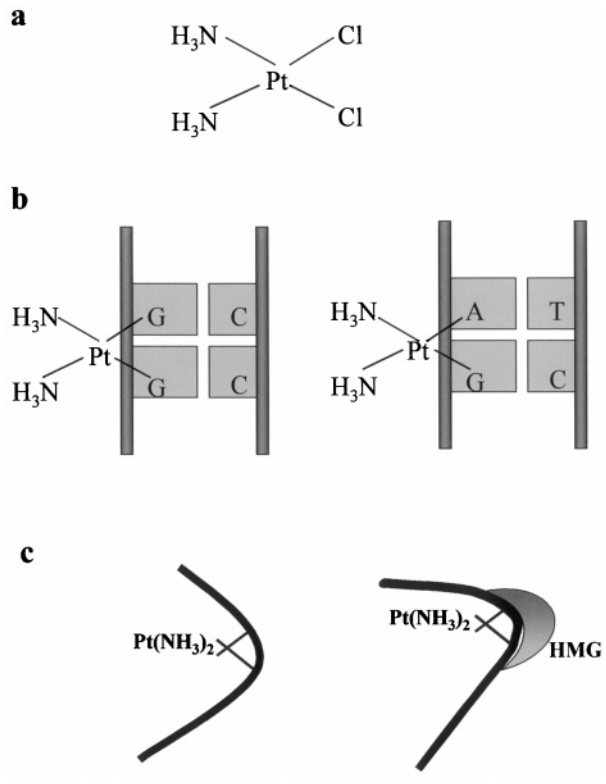

Figure 1. The platinum-DNA cross-link and HMG-DNA interactions. (*a*) In the cells, cisplatin is converted into a charged electrophilic compound that reacts with DNA. (*b*) The major adducts formed are intrastrand d(GpG) and d(ApG) cross-links. (*c*) These cross-links bend and unwind the DNA helix, and the altered structure attracts HMG-domain proteins.

Cisplatin-induced DNA adducts include protein-DNA cross-links, DNA monoadducts, and interstrand as well as intrastrand cross-links [6, 7]. Among these adducts, the vast majority consists of 1,2-d(GpG) and -d(ApG) cross-links formed between neighbouring purine bases (fig. 1b). Carboplatin has two amine groups in a cis configuration and forms DNA adducts similar to those of cisplatin. However, carboplatin is more stable and less reactive than cisplatin because the 1,1-cyclobutanedicarboxylato group in carboplatin is much less labile than the chloride in *cis*-DDP. As a result, the aquation reactions proceed more slowly in carboplatin than in cisplatin [8].

Formation of the 1,2 intrastrand *cis*-[$Pt(NH_3)_2$-d(GpG)] cross-link (G ^ G) in a double-stranded DNA helix has important structural consequences. The modified DNA fragments are bent, as was shown in solution by nuclear magnetic resonance (NMR) [9] and by X-ray crystallography [10]. The DNA is strongly kinked at a hydrophobic notch created at the platinum-DNA cross-link (fig. 1c).

Another potent nucleophile that reacts with cisplatin is glutathione. The reaction of glutathione with cisplatin forms a complex that is eliminated from the cell by an adenosine triphosphate (ATP)-dependent export pump. Thus, glutathione may contribute to cisplatin resistance by intercepting reactive platinum compounds before they reach the DNA. In the organism, cisplatin is additionally bound by metallothionein, a small protein involved in the detoxification of heavy metal ions. Whether binding to metallothionein plays any role in cisplatin resistance is not yet known, but certain cell lines resistant to heavy metals showed increases in metallothionein and became resistant to cisplatin (reviewed in [8]).

Cisplatin adducts are poorly repaired

In human cells, cisplatin adducts in DNA are repaired mainly by the nucleotide excision repair (NER) pathway, the system responsible for removal of ultraviolet (UV)-induced pyrimidine dimers [11–14]. Surprisingly, cisplatin adducts are repaired with very poor efficiency in cells, whereas in vitro they can be efficiently corrected [13]. In contrast, adducts generated by the less cytotoxic *trans*-DDP are much more efficiently repaired in vivo [12, 15]. This suggests that the cell's inability to repair the DNA lesions is important for the success of cisplatin as an anticancer drug. This view is further supported by the following observations. First, cell lines cultured from patients with xeroderma pigmentosum (a disease caused by genetic deficiencies in the NER pathway) are highly sensitive to cisplatin [16, 17]. Second, cancer tissues from patients whose tumours were clini-

cally resistant to cisplatin expressed enhanced levels of NER factors ERCC1 and XPA [18–20]. And finally, tumour cells hypersensitive to cisplatin were found deficient for these proteins [21].

Why are cisplatin adducts poorly repaired? The answer to this question apparently relies on a class of cellular proteins that bind to the cisplatin-induced 1,2 intrastrand cross-links due to an apparently fortuitous structural similarity with their natural binding sites [22]. These are the high-mobility-group (HMG) proteins, a class of abundant chromosome constituents defined by the presence of a common structural motif. The HMG domain consists of an L-shaped arrangement of three α helices with two independent DNA binding surfaces. Binding of a single HMG domain to DNA results in a distortion of the path of DNA through as much as 130° [23] (fig. 1c). Binding of HMG proteins to cisplatin adducts was shown to inhibit their repair [13, 24, 25]. Thus, a simple interpretation is that binding of cellular proteins to cisplatin-modified DNA prevents recognition of the lesion by the repair factors. Consistent with this 'repair shielding hypothesis', a yeast strain expressing the G^G-binding HMG-domain protein Ixr1 was twofold more sensitive to cisplatin and accumulated one-third more platinum-DNA lesions than a mutant strain lacking Ixr1 [26].

Although binding of HMG proteins to cisplatin-induced DNA lesions may inhibit nucleotide excision repair of the adducts, shielding of the G^G adduct from repair within the cell nucleus appears to be incomplete. This is suggested by the finding that whole-cell extracts containing endogenous HMG1 are able to repair G^G adducts, whereas addition of excess HMG1 protein inhibits repair [14, 24, 27]. Also, cells exposed to low doses of cisplatin and then transferred to drug-free medium can survive the treatment, most probably because the DNA lesions are successfully repaired.

Cisplatin adducts inhibit transcription

It is a long-standing observation that overall RNA synthesis is reduced in cells after treatment with DNA-damaging agents. In particular, the cisplatin-induced 1,2-G^G intrastrand cross-link was shown to block elongation of transcribing RNA polymerase II in vitro when it encountered the lesion [28–30]. Inhibition of transcription was further demonstrated in vivo. A two to threefold decrease in transcription level was observed when a cisplatin-modified β-galactosidase reporter gene was transfected into human or hamster cells [30]. Cisplatin was also shown to substantially reduce transcription from the mouse mammary tumour virus promotor stably transfected into mouse cells [31]. In addition, transcription of the glutathione *S*-transferase, studied by quantitative reverse transcription-polymerase chain reaction (RT-PCR), was inhibited to about 75% by cisplatin treatment of human glioblastoma cells [32].

Since NER occurs preferentially in actively transcribed genes and requires TFIIH, a general transcription factor for RNA polymerase II, it has been suggested that an RNA polymerase complex stalled on a DNA lesion triggers the assembly of the repair machinery leading to a faster correction of the damage when compared with the global genome repair [33, 34].

The coupling between transcription and NER was recently investigated in vitro using a competition assay in which transcriptionally active DNA was incubated with whole-cell extracts in the presence of cisplatin- or UV-damaged competitor DNA [35]. Under these conditions transcription from the viable template was inhibited. The factor responsible for mediating this inhibition was not TFIIH but, surprisingly, the TATA-binding protein TBP, a general transcription factor for all three RNA polymerases. TBP was shown to bind to the 1,2-intrastrand cisplatin adducts [35]. Drugs that cannot form these cross-links, such as transplatin or Dien [diethylenetriaminedichloroplatinum(II)], are neither recognised by TBP nor do they inhibit transcription [36]. Additionally, the minor 1,3-d(GpXpG) cisplatin-DNA cross-link that does not bend DNA is not recognised by TBP. Computer-assisted three-dimensional (3D)-structural analysis revealed a remarkable similarity between the crystal structure of the TATA-box/TBP complex, the structure of the 1,2-intrastrand *cis*-[Pt(NH_3)$_2$-d(GpG)] cross-link (G^G) and the structure of a UV-induced cyclobutane thymine dimer [35, 37]. Footprinting patterns then demonstrated that TBP protects equally four nucleotides upstream and six nucleotides downstream of either the TATA element or the G^G-cross-linked DNA [36]. Thus, similarly to HMG-domain proteins, TBP binds to the G^G-lesion due to shape complementarity with the damaged DNA structure.

Unexpectedly, the affinity of TBP for the 1,2-G^G adduct was shown to be about 200-fold stronger than for the TATA box. Furthermore, microinjection of additional TBP in living cells alleviates the reduction in RNA synthesis, suggesting that TBP is sequestered to the damaged DNA sites and consequently is unable to support transcription [35]. The idea that cisplatin-induced DNA lesions may hijack transcription factors and therefore inhibit transcription had been previously proposed following the discovery that G^G-lesion-binding HMG-domain proteins included the transcription factors UBF [38], SRY [39], LEF-1 [40] and Ixr1 [41]. In the case of SRY, kinetic data revealed that binding to the 1,2-G^G cross-link or to a putative target promotor sequence occurred with comparable affinity [42], further supporting the view that cisplatin-induced lesions compete with the natural SRY binding

sites. However, it remains questionable whether titration of transcription factors away from promoters is significant in vivo given the very high abundance of HMG proteins in the nucleus [22]. In this regard it is noteworthy that in yeast, cisplatin failed to affect the level of transcription of Cox5b, a gene regulated by the G ^ G-binding protein Ixr1 [41].

One of the HMG-domain proteins that binds 1,2-G ^ G-modified DNA, but not transplatin-DNA adducts, is the RNA polymerase I transcription factor 'upstream binding factor', UBF [38]. UBF binds to an upstream ribosomal DNA (rDNA) promotor control element and recruits the promotor selectivity factor (SL1) that appears to mediate communication with RNA polymerase I. Thus, if UBF fails to bind to the promotor, RNA polymerase I cannot initiate transcription.

The affinity of human UBF for the rDNA promotor is similar to that measured for the 1,2-G ^ G-intrastrand adduct, suggesting that UBF may be sequestered or hijacked by cisplatin-induced DNA-damaged sites [38]. A direct prediction from this model is that synthesis of rRNA should be inhibited in the presence of cisplatin. This was in fact recently confirmed using both in vivo and in vitro experimental approaches [43, 44].

Interestingly, cells treated with cisplatin and devoid of rRNA synthesis continue to incorporate RNA precursors throughout the nucleoplasm, indicating that the overall activity of RNA polymerase II is not significantly affected [43]. This immediately raises the question of whether rDNA is a preferential target for cisplatin action in vivo. In this regard it is important to note that in mammalian cells, repair of lesions induced by cisplatin or UV irradiation is significantly less efficient in rDNA than in RNA polymerase II-transcribed genes [45–47]. Moreover, cisplatin-induced G ^ G adducts compete with rDNA promoters for both UBF and TBP, two factors required for initiation of transcription by RNA polymerase I. Thus, if repair is inefficient, rDNA is expected to accumulate more lesions than genes transcribed by RNA polymerase II, and these abundant damaged sites will sequester two major RNA polymerase I transcription factors, therefore further contributing to inhibit transcription. This implies that disruption of rRNA synthesis may represent an important cellular consequence of cisplatin action. In fact, since rRNA synthesis is higher in proliferating and metabolically active cells [48], by blocking transcription of rDNA genes, cisplatin may be preferentially cytotoxic to rapidly dividing cancer cells and cells with very high rates of protein synthesis such as neurons. In this regard, it is noteworthy that in humans a major side effect of cisplatin treatment is a peripheral sensory neuropathy secondary to dorsal root ganglion involvement and that the affected neurons show significant nucleolar alterations [49].

Cisplatin adducts trigger apoptosis

Inhibition of DNA synthesis by cisplatin was initially claimed to be responsible for its antitumour activity [50]. However, subsequent studies showed that inhibition of DNA synthesis did not correlate with the sensitivity of different cell lines to the drug [51]. More recently it has been proposed that cisplatin kills cancer cells by triggering G2 cell-cycle arrest and apoptosis [8, 52]. Although it remains to be established whether the clinical success of cisplatin relies primarily on its ability to trigger apoptosis, two cellular proteins, p53 and p73, are known to induce cell-cycle arrest/apoptosis in response to DNA damage, and very recent results suggest that cisplatin can induce two parallel death-response pathways, one dependent on p53 and the other on p73 [53]. The tumor-suppressor protein p53 is a potent activator of apoptosis, and p53-deficient cancer cells are less responsive to cisplatin therapy [54, 55]. However, there is evidence that cells can be killed by cisplatin in a p53-independent manner [56], implying the presence of other apoptotic pathways. One such alternative pathway may involve the product of a *p53*-related gene, *p73*. The *p73* gene encodes a family of proteins with the ability to induce apoptosis [57]. Cisplatin induces an activation of the c-Abl tyrosine kinase, an essential mediator of cell-cycle arrest in response to DNA damage [58, 59], and c-Abl potentiates the proapoptotic activity of p73 [53, 60, 61]. These results suggest that DNA damage signals are channelled through c-Abl to p73.

Other likely regulators of the cisplatin-induced death response include expression of the Bcl-2 family of proteins [62], activation of stress-kinase cascades [63, 64] and telomere loss [65].

In particular, a direct role of mismatch repair proteins in coupling cisplatin damage to an apoptotic response is currently well established [66]. The main experimental support to this model can be summarised as follows. First, the mismatch repair protein complex can recognise and bind to the 1,2-G ^ G adducts [67–69]. Second, cells defective for mismatch repair function become more resistant to G2 arrest and apoptosis in the presence of cisplatin [70–73]. Lastly, the activation of p73 in response to cisplatin is not seen in cells unable to carry out mismatch repair [53].

Recently, it was reported that inactivation of mismatch repair genes in yeast led to increased resistance to cisplatin [74]. However, no change in drug resistance was observed after inactivation of these genes on rad52 or rad1 mutant strains, which are defective in mitotic recombination and removal of unpaired DNA single strands. Interestingly, the human Ku protein (involved in double-stranded break repair and recombination) also recognises the 1,2-G ^ G adducts [75]. Taken to-

gether, these data suggest that proteins of the homologous recombinatorial repair pathway (i.e. the system that repairs double-strand breaks arising on chromosomes during DNA replication) also participate in the recognition of cisplatin-induced DNA lesions.

Although DNA replication tends to be inhibited in the presence of cisplatin, some G ^ G intrastrand cross-links may be bypassed by DNA polymerase before replication comes to a complete stop [14, 76–78]. As a result of this so-called translesion DNA synthesis, random nucleotides will be incorporated opposite to the lesion [79]. Consequently, the new helix will contain a mismatch, and this will be recognised by the DNA mismatch repair machinery. Binding of the mismatch repair proteins to the DNA would prevent the NER system from becoming active by simple physical competition and thus would inhibit repair of the lesion [80]. In contrast with NER, which is likely to act independently of the cell cycle, the mismatch repair system probably acts mainly during the postreplicative phase, when the highest concentration of mispairs is expected to be present in the newly synthesised DNA. Thus, rapidly proliferating cells are more prone to have futile repair attempts which could eventually trigger cell death. One prediction from this model is that inactivation of mismatch repair proteins should allow, first, a more efficient repair of the adducts by the NER system (with consequent resistance to cisplatin) and, second, a hypermutability phenotype due to inability to detect mismatch mutations that occur either spontaneously or during translesion synthesis. Consistent with this view, cisplatin-resistant ovarian cell lines were shown to be defective in strand-specific mismatch repair and to acquire a greatly increased rate of mutation at microsatellite sequences [70]. Moreover, resistant cell lines which acquire a microsatellite instability phenotype also loose p53 function, thus contributing to the inability to undergo cisplatin-induced apoptosis [54, 70].

Conclusions and prospects

In summary, cisplatin induces DNA adducts that are poorly repaired by the NER system. Abortive repair attempts are recognised by mismatch repair proteins and activate a programmed cell death response. Interaction of cisplatin with DNA alters its architecture in a way that mimics the natural recognition motif of several nuclear DNA-binding proteins, including HMG proteins and TBP. These proteins compete with the NER machinery for binding to cisplatin adducts, therefore contributing to their inefficient repair. Ribosomal DNA appears to be a major target for cisplatin action, and this may be explained by the fact that in mammalian cells RNA polymerase II-transcribed genes are preferentially repaired by NER. This observation implies that the nucleolus may be involved in triggering apoptosis, but future studies are needed to address the coupling between cisplatin induced DNA damage and cell death response.

A further issue that will still attract much attention is the problem of resistance to cisplatin. In the case of ovarian cancer, 20–30% of tumours are intrinsically resistant to cisplatin, and relapses of initially responsive tumours may acquire resistance [81]. The challenge here is to develop new platinum-based cytostatic drugs able to circumvent resistance. Promising, but still preliminary results have been obtained with oxaliplatin [1,2-diaminocyclohexane platinum (II) oxalate], a drug that produces DNA adducts similar to cisplatin but efficiently kills cells with deficiencies in mismatch repair and increases in replicative bypass [82]. In this light, recent developments in determining how cellular proteins bind to cisplatin-modified DNA [83, 84] are likely to help the rational design of novel classes of platinum anticancer drugs.

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1 Rosenberg B., van Camp L. and Krigas T. (1965) Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. Nature **205:** 698–699
2 Sherman S. E. and Lippard S. J. (1987) Structural aspects of platinum anticancer drug interactions with DNA. Chem. Rev. **86:** 1153–1181
3 Muggia F. M. (1991) Cisplatin update. Semin. Oncol. **18:** 1–4
4 Leng M., and Barbec V. (1994) DNA adducts of cisplatin, transplatin and platinum-intercalating drugs. In: DNA Adducts: Identification and Biological Significance, pp. 339–348, Hemminki K. et al. (eds), IARC Scientific Publications no. 125, International Agency for Research on Cancer, Lyons, France
5 Loehrer P. J. and Einhorn L. H. (1984) Cisplatin. Annals Int. Med. **100:** 704–713
6 Pinedo H. M. and Schornagel J. H. (1996) Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy, Plenum, New York
7 Yang D. Z. and Wang A. H. J. (1997) Structural studies of interactions between anticancer platinum drugs and DNA. Prog. Biophys. Mol. Biol. **66:** 81–111
8 Chu G. (1994) Cellular responses to cisplatin. J. Biol. Chem. **269:** 787–790
9 Gelasco A. and Lippard S. J. (1998) NMR solution structure of a DNA dodecamer duplex containing a cis-diammineplatinum(II) d(GpG) intrastrand cross-link, the major adduct of the anticancer drug cisplatin. Biochemistry **37:** 9230–9239
10 Takahara P. M., Rosenzweig A. C., Frederick C. A. and Lippard S. J. (1995) Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. Nature **377:** 649–652
11 Lindahl T., Karran P. and Wood R. D. (1997) DNA excision repair pathways. Curr. Opin. Genet. Dev. **7:** 158–169
12 Zample D. B. and Lippard S. J. (1995) Cisplatin and DNA repair in cancer chemotherapy. Trends Biochem. Sci. **20:** 435–439

13 Zamble D. B., Mu D., Reardon J. T., Sancar A. and Lippard S. J. (1996) Repair of cisplatin-DNA adducts by the mammalian excision nuclease. Biochemistry **35:** 10004–10013
14 Moggs J. G., Szymkowski D. E., Yamada M., Karran P. and Wood R. D. (1997) Differential human nucleotide excision repair of paired and mispaired cisplatin-DNA adducts. Nucleic Acids Res. **25:** 480–491
15 Ciccarelli R. B., Solomon M. J., Varshavsky A. and Lippard S. J. (1985) In vivo effects of cis- and trans-diamminedichloroplatinum(II) on SV40 chromosomes: differential repair, DNA-protein cross-linking and inhibition of replication. Biochemistry **24:** 7533–7540
16 Plooy A. C., Fichtinger-Schepman A. M., Schutte H. H., van Dijk M. and Lohman P. H. (1985) The quantitative detection of various Pt-DNA-adducts in Chinese hamster ovary cells treated with cisplatin: application of immunochemical techniques. Carcinogenesis **6:** 561–566
17 Dijt F. J., Fichtinger-Schepman A. M., Berends F. and Reedijk J. (1988) Formation and repair of cisplatin-induced adducts to DNA in cultured normal and repair-deficient human fibroblasts. Cancer Res. **48:** 6058–6062
18 Dabholkar M., Vionnet J., Bostick-Bruton F., Yu J. J. and Reed E. (1994) Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. J. Clin. Invest. **94:** 703–708
19 States J. C. and Reed E. (1996) Enhanced XPA mRNA levels in cisplatin-resistant human ovarian cancer is not associated with XPA mutations or gene amplification. Cancer Lett. **108:** 233–237
20 Li Q., Ding L., Yu J. J., Mu C., Tsang B., Bostick-Bruton F. et al. (1998) Cisplatin and phorbol ester independently induce ERCC-1 protein in human ovarian carcinoma cells. Int. J. Oncol. **13:** 987–992
21 Koberle B., Masters J. R. W., Hartley J. A. and Wood R. D. (1999) Defective repair of cisplatin-induced DNA damage caused by reduced XPA protein in testicular germ cell tumours. Curr. Biol. **9:** 273–276
22 Zlatanova J., Yaneva J. and Leuba S. H. (1998) Proteins that specifically recognize cisplatin-damaged DNA: a clue to anticancer activity of cisplatin. FASEB J. **12:** 791–799
23 Bianchi M. E. and Beltrame M. (1998) Flexing DNA: HMG-box proteins and their partners. Am. J. Hum. Genet. **63:** 1573–1577
24 Huang J. C., Zamble D. B., Reardon J. T., Lippard S. J. and Sancar A. (1994) HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. Proc. Natl. Acad. Sci. USA **91:** 10394–10398
25 Fleck O., Kunz C., Rudolph C. and Kohli J. (1998) The high mobility group domain protein Cmb1 of *Schizosaccharomyces pombe* binds cytosines in base mismatches and opposite chemically altered guanines. J. Biol. Chem. **273:** 30398–30405
26 Brown S. J., Kellett P. J. and Lippard S. J. (1993) Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin. Science **261:** 603–605
27 Jones S. L., Hickson I. D., Harris A. L. and Harnett P. R. (1994) Repair of cisplatin-DNA adducts by protein extracts from human ovarian carcinoma. Int. J. Cancer **59:** 388–393
28 Corda Y., Job C., Anin M.-F., Leng M. and Job D. (1991) Transcription by eucaryotic and procaryotic RNA polymerases of DNA modified at a d(GG) or a d(AG) site by the antitumor drug cis-diammindichloroplatinum(II). Biochemistry **30:** 222–230
29 Lemaire M. A., Schwartz A., Rahmouni A. R. and Leng M. (1991) Interstrand cross-links are preferentially formed at the d(GC) sites in the reaction between cis-diamminedichloroplatinum (II) and DNA. Proc. Natl. Acad. Sci. USA **88:** 1982–1985
30 Mello J. A., Lippard S. J. and Essigmann J. M. (1995) DNA adducts of cis-diamminedichloroplatinum(II) and its trans isomer inhibit RNA polymerase II differentially in vivo. Biochemistry **34:** 14783–14791
31 Mymryk J. S., Zaniewski E. and Archer T. K. (1995) Cisplatin inhibits chromatin remodeling, transcription factor binding and transcription from the mouse mammary tumor virus promoter in vivo. Proc. Natl. Acad. Sci. USA **92:** 2076–2080
32 Brandt T. Y. and Ali-Osman F. (1997) Detection of DNA damage in transcriptionally active genes by RT-PCR and assessment of repair of cisplatin-induced damage in the glutathione S-transferase-pi gene in human glioblastoma cells. Toxicol. Appl. Pharmacol. **143:** 22–29
33 Friedberg E. C. (1996) Relationships between DNA repair and transcription. Annu. Rev. Biochem. **65:** 15–42
34 van Gool A. J., Van der Horst G. T. J., Citterio E. and Hoeijmakers J. H. J. (1997) Cockayne syndrome: defective repair of transcription. EMBO J. **16:** 4155–4162
35 Vichi P., Coin F., Renaud J.-P., Vermeulen W., Hoeijmakers J. H. J., Moras D. et al. (1997) Cisplatin- and UV-damaged DNA lure the basal transcription factor TFIID/TBP. EMBO J. **16:** 7444–7456
36 Coin F., Frit P., Viollet B., Salles B. and Egly J. M. (1998) TATA binding protein discriminates between different lesions on DNA, resulting in a transcription decrease. Mol. Cell. Biol. **18:** 3907–3914
37 Vassylyev D. G., Kashiwagi T., Mikami Y., Ariyoshi M., Iwai S., Ohtsuka E. et al. (1995) Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA substrate: structural basis for damaged DNA recognition. Cell **83:** 773–782
38 Treiber D. K., Zhai X., Jantzen H.-M. and Essigmann J. M. (1994) Cisplatin-DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human upstream binding factor). Proc. Natl. Acad. Sci. USA **91:** 5672–5676
39 Werner M. H., Huth J. R., Gronenborn A. M. and Cloe G. M. (1995) Molecular basis of human 46X,Y sex reversal revealed from the three-dimensional solution structure of the human SRY-DNA complex. Cell **81:** 705–714
40 Love J. J., Li X., Case D. A., Giese K., Grosschedl R. and Wright P. E. (1995) Structural basis for DNA bending by the architectural transcription factor LEF-1. Nature **376:** 791–795
41 McA'Nulty M. M., Whitehead J. P. and Lippard S. J. (1996) Binding of Ixr1, a yeast HMG-domain protein, to cisplatin-DNA adducts in vitro and in vivo. Biochemistry **35:** 6089–6099
42 Trimmer E. E., Zamble D. B., Lippard S. J. and Essigmann J. M. (1998) Human testis-determining factor SRY binds to the major DNA adduct of cisplatin and a putative target sequence with comparable affinities. Biochemistry **37:** 352–362
43 Jordan P. and Carmo-Fonseca M. (1998) Cisplatin inhibits synthesis of ribosomal RNA in vivo. Nucleic Acids Res. **26:** 2831–2836
44 Zhai X., Beckmann H., Jantzen H. M. and Essigmann J. M. (1998) Cisplatin-DNA adducts inhibit ribosomal RNA synthesis by hijacking the transcription factor human upstream binding factor. Biochemistry **37:** 16307–16315
45 Vos J.-M. H. and Wauthier E. (1991) Differential introduction of DNA damage and repair in mammalian genes transcribed by RNA polymerases I and II. Mol. Cell. Biol. **11:** 2245–2252
46 Christians F. C. and Hanawalt P. C. (1993) Lack of transcription-coupled repair in mammalian ribosomal RNA genes. Biochemistry **32:** 10512–10518
47 Fritz L. K. and Smerdon M. J. (1995) Repair of UV damage in actively transcribed ribosomal genes. Biochemistry **34:** 13117–13124
48 Moss T. and Stefanovsky V. Y. (1995) Promotion and regulation of ribosomal transcription in eukaryotes by RNA polymerase I. Prog. Nucleic Acid Res. Mol. Biol. **50:** 25–66
49 Cece R., Petrucciolo M. G., Cavaletti G., Barajon I. and Tredici G. (1995) An ultrastructural study of neuronal changes in dorsal root ganglia of rats after chronic cisplatin administration. Histol. Histopathol. **10:** 837–845

50 Pinto A. L. and Lippard S. J. (1985) Sequence-dependent termination of in vitro DNA synthesis by cis- and trans-diamminedichloroplatinum (II). Proc. Natl. Acad. Sci. USA **82:** 4616–4619

51 Sorenson C. M. and Eastman A. (1988) Influence of cis-diamminedichloroplatinum(II) on DNA synthesis and cell cycle progression in excision repair proficient and deficient Chinese hamster ovary cells. Cancer Res. **48:** 6703–6707

52 Allday M. J., Inman G. J., Crawford D. H. and Farrell P. J. (1995) DNA damage in human B cells can induce apoptosis, proceeding from G1/S when p53 is transactivation competent and G2/M when it is transactivation defective. EMBO J. **14:** 4994–5005

53 Gong J, Costanzo A., Yang H.-Q., Melino G., Kaelin W. G. Jr, Levrero M. et al. (1999) The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature **399:** 806–809

54 Anthoney D. A., McIlwrath A. J., Gallagher W. M., Edlin A. R. M. and Brown R. (1996) Microsatellite instability, apoptosis and loss of p53 function in drug-resistant tumour cells. Cancer Res. **56:** 1374–1381

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

56 Zamble D. B., Jacks T. and Lippard S. J. (1998) p53-dependent and -independent responses to cisplatin in mouse testicular teratocarcinoma cells. Proc. Natl. Acad. Sci. USA **95:** 6163–6168

57 Jost C. A., Mrin M. C. and Kaelin W.G. Jr (1999) p73 is a human p53-related protein that can induce apoptosis. Nature **389:** 191–194

58 Kharbanda S., Ren R., Pandey P., Shafman T. D., Feller S. M., Weichelbaum R. et al. (1995) Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents. Nature **376:** 785–788

59 Liu Z. G., Baskaran R., Lea-Chou E. T., Wood L. D., Chen Y., Karin M. et al. (1996) Three distinct signalling responses by murine fibroblasts to genotoxic stress. Nature **384:** 273–276

60 Agami R., Blandino G., Oren M. and Shaul Y. (1999) Interaction of c-Abl and p73α and their collaboration to induce apoptosis. Nature **399:** 809–813

61 Yuan Z., Shioya H., Ishiko T., Sun X., Gu J., Huang Y. Y. et al. (1999) p73 is regulated by tyrosine-kinase c-Abl in the apoptotic response to DNA damage. Nature **399:** 814–817

62 Strasser A., Harris A. W., Jacks T. and Cory S. (1994) DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. Cell **79:** 329–339

63 Zanke B. W., Boudreau K., Rubie E., Winnettt E., Tibbles L. A., Zon L. et al. (1996) The stress-activated protein kinase pathway mediates cell death following injury induced by cis-platinum, UV irradiation or heat. Curr. Biol. **6:** 606–613

64 Sanchez-Perez I., Murguia J. R. and Perona R. (1998) Cisplatin induces a persistent activation of JNK that is related to cell death. Oncogene **16:** 533–540

65 Ishibashi T. and Lippard S. J. (1998) Telomere loss in cells treated with cisplatin. Proc. Natl. Acad. Sci. USA **95:** 4219–4223

66 Strathdee G., MacKean M. J., Illand M. and Brown R. (1999) A role for methylation of the *hMLH1* promoter in loss of hMLH1 expression and drug resistance in ovarian cancer. Oncogene **18:** 2335–2341

67 Mello J. A., Acharya S., Fishel R. and Essigmann J. M. (1996) The mismatch-repair protein hMSH2 binds selectively to DNA adducts of the anticancer drug cisplatin. Chem. Biol. **3:** 579–589

68 Duckett D. R., Drummond J. T., Murchie A. I., Reardon J. T., Sancar A., Lilley D. M. et al. (1996) Human MutSα recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylthymine or the cisplatin-d(GpG) adduct. Proc. Natl. Acad. Sci. USA **93:** 6443–6447

69 Yamada M., O'Regan E., Brown R. and Karran P. (1997) Selective recognition of a cisplatin-DNA adduct by human mismatch repair proteins. Nucleic Acids Res **25:** 491–496

70 Drummond J. T., Anthoney A., Brown R. and Modrich P. (1996) Cisplatin and adriamycin resistance are associated with MutLα and mismatch repair deficiency in an ovarian tumour cell line. J. Biol. Chem. **271:** 19645–19648

71 Aebi S., Kurdi-Haidar B., Gordon R., Cenni B., Zheng H., Fink D. et al. (1996) Loss of DNA mismatch repair in acquired resistance to cisplatin. Cancer Res. **56:** 3087–3090

72 Vaisman A., Varchenko M., Umar A., Kunkel T. A., Risinger J. I., Barrett J. C. et al. (1998) The role of hMLH1, hMSH3 and hMSH6 defects in cisplatin and oxaliplatin resistance: correlation with replicative bypass of platinum-DNA adducts. Cancer Res. **58:** 3579–3585

73 Fink D., Nebel S., Norris P. S., Baergen R. N., Wilcynski S. P., Costa M. J. et al. (1998) Enrichment for DNA mismatch repair-deficient cells during treatment with cisplatin. Int. J. Cancer **77:** 741–746

74 Durant S. T., Morris M. M., Illand M., McKay H. J., McCormick C., Hirst G. L. et al. (1999) Dependence on RAD52 and RAD1 for anticancer drug resistance mediated by inactivation of mismatch repair genes. Curr. Biol. **9:** 51–54

75 Turchi J. J. and Henkels K. M. (1996) Human Ku autoantigen binds cisplatin-damaged DNA but fails to stimulate human DNA-activated protein kinase. J. Biol. Chem. **271:** 13861–13867

76 Comess K. M., Burstyn J. N., Essigmann J. M. and Lippard S. J. (1992) Replication inhibition and translesion synthesis on templates containing site-specifically placed cis-diamminedichloroplatinum(II) DNA adducts. Biochemistry **31:** 3975–3990

77 Pillaire M. J., Hoffmann J. S., Defais M. and Villani G. (1995) Replication of DNA containing cisplatin lesions and its mutagenic consequences. Biochimie **77:** 803–807

78 Hoffmann J. S., Pillaire M. J., Lesca C., Burnouf D., Fuchs R. P., Defais M. et al. (1996) Fork-like DNA templates support bypass replication of lesions that block DNA synthesis on single-stranded templates. Proc. Natl. Acad. Sci. USA **93:** 13766–13769

79 Yarema K. J., Lippard S. J. and Essigmann J. M. (1995) Mutagenic and genotoxic effects of DNA adducts formed by the anticancer drug cis-diamminedichloroplatinum(II). Nucleic Acids Res. **23:** 4066–4072

80 Marra G. and Schär P. (1999) Recognition of DNA alterations by the mismatch repair system. Biochem. J. **388:** 1–13

81 Neijt J. P. (1997) Textbook of Medical Oncology, Cavalli F., Hansen H. H. and Kaye S. B. (eds), Martin Dunitz, London

82 Raymond E., Faivre S., Woynarowski J. M. and Chaney S. G. (1998) Oxaliplatin: mechanism of action and antineoplastic activity. Sem. Oncol. **2**(Suppl. 5)**:** 4–12

83 Ohndorf U.-M., Rould M. A., He Q., Pabo C. O. and Lippard S. J. (1999) Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. Nature **399:** 708–712

84 Allain F. H.-T., Yen Y. M., Masse J. E., Schultze P., Dieckmann T., Johnson R. C. et al. (1999) Solution structure of the HMG-protein NHP6A and its interaction with DNA reveals the structural determinants for non-sequence-specific binding. EMBO J. **18:** 2563–2579