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## Advances in Brief

# Transcription-coupled Nucleotide Excision Repair as a Determinant of Cisplatin Sensitivity of Human Cells[1]

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## Abstract

The resistance of tumor cells to chemotherapeutic agents, such as cisplatin, is an important problem to be solved in cancer chemotherapy. One of the mechanisms associated with cisplatin resistance is nucleotide excision repair (NER). There are two pathways in NER, transcription-coupled NER (TC-NER) and global genome NER (GG-NER). Here, we report that TC-NER-deficient cells [xeroderma pigmentosum group A (XP-A), XP-D, XP-F, XP-G, Cockayne syndrome group A (CS-A), and CS-B] are hypersensitive to cisplatin irrespective of their GG-NER status, and that gene complementation with *XPA* and *XPD* increases resistance to cisplatin. By contrast, XP-C cells with selective defect in GG-NER but with normal TC-NER have normal resistance to cisplatin. XPC complementation had no effect on cisplatin antiproliferative activity. We propose that one of the pathways related to cisplatin response is TC-NER, not GG-NER.

## Introduction

Cisplatin is widely used for the treatment of various malignant tumors, including testicular, ovarian, head and neck, and lung (1). The basis for the therapeutic effectiveness of cisplatin is not fully understood, but the activity of cisplatin against tumor cells is thought to be mediated through the formation of cisplatin-DNA adducts that block replication or inhibit transcription. Cisplatin forms primarily 1,2-intrastrand cross-links between adjacent purines in DNA and also introduces DNA 1,3-intrastrand cross-links, interstrand cross-links, and monoadducts (2, 3). Cisplatin-based chemotherapies are remarkably effective in testicular cancers, and most patients are cured with combination regimens including cisplatin, bleomycin, and etoposide (3). Cisplatin in association with Taxol is also very effective in ovarian carcinoma, with an initial response rate of ~50% (4). However, only 20–30% of these initial responders have a durable response, and most patients relapse (4). Thus, a common problem for cisplatin treatments is intrinsic, as well as acquired, resistance. Resistance to cisplatin is potentially multifactorial (3) and has been attributed to decreased drug accumulation, enhanced cellular detoxification by enhanced levels of glutathione or metallothionein, and enhanced DNA repair.

NER[3] is the main mechanism for removing cisplatin adducts. NER has been elucidated at the molecular level in recent years, and it is now possible to follow step by step the repair of adducts and cross-

links on adjacent DNA bases (2, 3). The NER molecular machinery includes well-defined proteins that are mutated in XP and CS patients (5–7). NER can be subdivided into two pathways: TC-NER and GG-NER. TC-NER repairs transcription-blocking lesions in transcribed DNA strands of active genes (7), whereas the repair of lesions in the nontranscribed strand of active genes and nontranscribing genome (≈70% of the genome; Ref. 8) is carried out by the GG-NER pathway. Each of these subpathways consists of specific repair factors. For TC-NER, RNA polymerase II is considered the sensor for DNA damage. When transcribing RNA polymerase II encounters the lesion, two transcription-coupled repair-specific factors, CSA and CSB, are implicated for the activation of the common NER molecular pathway. For GG-NER, the XPC/hHR23B dimer engages the common NER pathway. In the common NER pathway, TFIIH and XPG bind to the DNA around the lesion. The basal transcription factor, TFIIH, contains two helicases, XPB and XPD, which open approximately a 30-base-long DNA segment around the damage. This open intermediate is stabilized by replication protein A and XPA. The DNA strand that contains the damaged base(s) is excised by the two NER endonucleases, XPG and XPF/ERCC1. XPG cleaves the damaged DNA strand 3′ from the lesion, and XPF/ERCC1 cleaves the damaged strand 5′ from the DNA lesion. The resulting gap is filled in by DNA polymerase $\delta$ or $\varepsilon$ in the presence of replication factors (6, 9).

We reported recently the selective role of TC-NER in the anticancer activity of the novel anticancer drug, Et743 a sequence-specific guanine N2 alkylator (10). We proposed that Et743 kills cancer cells selectively in their attempt to repair transcription-blocking lesions. This finding raises the possibility that TC-NER might be a critical determinant for the antitumor activity of other anticancer drugs. The present study demonstrates the importance of TC-NER (and the apparent irrelevance of GG-NER in this context) for cellular response to cisplatin. These findings suggest the value of measuring TC-NER defects in tumors as a predictor of cellular response to cisplatin.

## Materials and Methods

The panel of cell lines used in the present study is listed in Table 1. Two sets of SV40-transformed XP-C and XP-C-corrected cells, XP44RO and XP44RO+XPC, and XP4PA-SV-EB and XP4PA-SE2, were generated as described previously (11, 12). Other cell lines were purchased from the Coriell Institute for Medical Research (Camden, NJ). Cells were cultured in DMEM containing 10% heat-inactivated FBS. The IC$_{50}$ for cisplatin was determined in each cell line by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. In brief, cells were seeded into 96-well microplates at 1000 cells/100 $\mu$l in each well. Two days later after seeding, the cells were treated without or with the indicated concentrations of cisplatin. After 72 h incubation, 10 $\mu$l of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Chemical Co., Louis, MO) solution (5 mg/ml) were added to the each well. Four h later, the medium was aspirated, and 100 $\mu$l of DMSO (Sigma Chemical Co.) were added to each well. The absorbance of each well (wavelength, 550–650 nm) was measured with a microplate reader (Emax; Molecular Devices Corp., Sunnyvale, CA). The survival of cells at each concentra-

Table 1 *Characteristics and IC$_{50}$s for cisplatin of primary and transformed cell lines used in the study*

| Cell | Phenotype | TC-NER | GG-NER | Primary or transformed | IC$_{50}$ ($\mu$M)$^a$ |
|------|-----------|--------|--------|------------------------|------------------------|
| AG06239 | Normal | + | + | Primary | 35 |
| AG13153 | Normal | + | + | Primary | 30 |
| GM01856 | CS-A | − | + | Primary | 0.9 |
| GM01098 | CS-B | − | + | Primary | 0.4 |
| GM04314 | XP-A | − | − | Primary | 1.1 |
| GM16370 | XP-C | + | − | Primary | 21 |
| GM11613 | XP-D | − | − | Primary | 1.2 |
| AG08803 | XP-G | − | − | Primary | 0.8 |
| GM00637 | Normal | + | + | Transformed | 5.8 |
| GM16095 | CS-B | − | + | Transformed | 0.4 |
| GM04312 | XP-A | − | − | Transformed | 1.3 |
| GM15876 | XP-A-Corrected | + | + | Transformed | 8.9 |
| XP4PA-SV-EB | XP-C | + | − | Transformed | 3.5 |
| XP44RO | XP-C* | + | − | Transformed$^b$ | 8.2 |
| XP4PA-SE2 | XP-C-Corrected | + | + | Transformed | 4.5 |
| XP44RO+XPC | XP-C*-Corrected | + | + | Transformed$^b$ | 8.8 |
| GM08207 | XP-D | − | − | Transformed | 0.4 |
| GM15877 | XP-D-Corrected | + | + | Transformed | 3.7 |
| GM08437 | XP-F | − | − | Transformed | 0.8 |
| GM14930 | XP-G | − | − | Transformed | 0.4 |

$^a$ IC$_{50}$s (50% inhibitory concentrations of cell growth) to cisplatin were determined by MTT assay.
$^b$ XP44RO and XP44RO+XPC cells were derived from melanoma of an XP-C patient (11). Other transformed cell lines were SV40-transformed.

tion of drug was expressed as the percentage ratio of the absorbance of cells treated with different concentrations of cisplatin to the mean absorbance of the untreated cells. Measurements were performed in triplicate. The IC$_{50}$ for cisplatin for each cell line was calculated from the dose-survival curves.

Numerical data were expressed as means ($\pm$SE). The statistical significance of parameters associated with IC$_{50}$s in relation to the independent effects of TC-NER proficient/deficient, GG-NER proficient/deficient, and primary/transformed status was assessed by multiple regression analysis (StatView 5.0; SAS Institute, Cary, NC). All *P*s were two-sided. A value of $P < 0.05$ was considered statistically significant.

## Results

Cell lines derived from patients with known molecular deficiencies in each of the NER molecular subpathways were used to examine cisplatin activity (Fig. 1). We tested primary, SV40-transformed, and tumor cell lines. For the XP-A, XP-C, and XP-D cell lines, we were able to compare drug response in complemented (corrected) cells (see Table 1).

Fig. 2*A* shows that molecular deficiencies in primary fibroblasts that are deficient in both GG-NER and TC-NER pathways (XP-A, XP-D, XP-F, and XP-G) confer marked hypersensitivity to cisplatin compared with normal primary fibroblasts.

SV40 transformation increased the sensitivity to cisplatin in normal cells. However, SV40-transformed fibroblasts from XP-A, XP-D, XP-F, or XP-G patients were hypersensitive to cisplatin compared with SV40-transformed normal cells (Fig. 2*B*). Complementation of the XP deficiencies by stable transfection of the deficient *XP* genes restored a normal phenotype for both the XP-A and XP-D cells (Fig. 2*B*). These results demonstrate that any one deficiency in the common NER pathway (XP-A, XP-D, XP-F, or XP-G) confers marked hypersensitivity to cisplatin.

To explore the contribution of the GG-NER and TC-NER pathways, experiments were performed with CS cells (selectively deficient for TC-NER) and XP-C cells (selectively deficient for GG-NER). Fig. 2*C* shows that both the primary CS-A and CS-B cells were hypersensitive to cisplatin, whereas primary XP-C fibroblasts had normal sensitivity to cisplatin, and responded similarly to normal fibroblasts (AG06239 and A13153; shaded area in Fig. 2*A* and 2*C*). The SV40-transformed cells (Fig. 2*D*) showed similar results, with CS-B cells being hypersensitive to cisplatin, whereas two sets of XP-C cells

showed relative resistance to cisplatin and similar response to SV40-transformed normal fibroblasts (GM637) cells. Furthermore, correction of the XPC defect in both XP-C cell lines failed to affect cisplatin response (Fig. 2*D*), although the original defects in UV-induced DNA damage repair were corrected (12, 13).

Table 1 summarizes the results obtained in the cell lines examined and the relationship to the NER status of the cells. Multiple regression analysis revealed that TC-NER status was a highly significant factor affecting the responses of cells to cisplatin, and that the GG-NER status of the cells was not associated with differential activity of cisplatin (Table 2). Our analysis also demonstrated that transformed cells were significantly more sensitive to cisplatin than primary cells (Table 2).

## Discussion

In the present study, we tested the cellular determinants of response and resistance to cisplatin using a panel of human cell lines with known genetic deficiencies in NER and with cell lines complemented with *XPA*, *XPC*, or *XPD* genes and found that the cytotoxic activity of cisplatin depends on the TC-NER status of the cells, not on their GG-NER status.

Cisplatin-DNA adducts are considered to be the main lesions that cause cellular death and therefore tumor response. The efficiency of removal of the cisplatin-DNA adducts by NER is assumed to be one of the determinants of cisplatin resistance. Recent studies have revealed that ERCC1 or ERCC1/XPF complexes (which are both essential for NER) are overexpressed in cisplatin-resistant cells (14). Enhanced activation of other NER factors (such as XPA) has also been proposed to account for cisplatin resistance (3). Conversely, NER mutant cells are hypersensitive to cisplatin (15). Furthermore, testicular tumor cells, which are highly responsive to cisplatin, have been found deficient for NER because of low levels of XPA and ERCC1/XPF (16).

Of the two NER pathways, the multiple regression analysis (Table 2) revealed that only TC-NER is relevant for the antiproliferative activity of cisplatin. Thus, the transcribing genome, which represents ~30% of any given cell's genome [if one includes both the introns and exons (8)], appears to be of primary importance for cisplatin activity. Conversely, we found that NER deficiency in the nontranscribing genome had no effect on cisplatin activity. The three XP-C cell lines used in the present study responded to cisplatin similarly to normal fibroblast cell lines. Moreover, we found that complementation of XPC in two different XP-C cell lines had no effect on the cellular response to cisplatin. These observations imply that XPC (the GG-NER initiating factor) is dispensable for the repair of cisplatin-
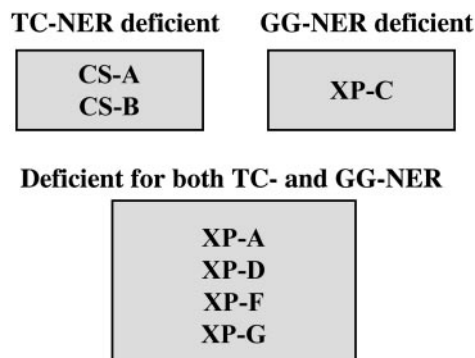


Fig. 1. Schematic representation of the NER-deficient cells tested in the present study. CS cells (CS-A and CS-B) are specifically deficient for the TC-NER pathway. XP-C cells are specifically deficient for the GG-NER pathway. XP-A, XPD, XP-F, and XP-G cells are deficient for both pathways.
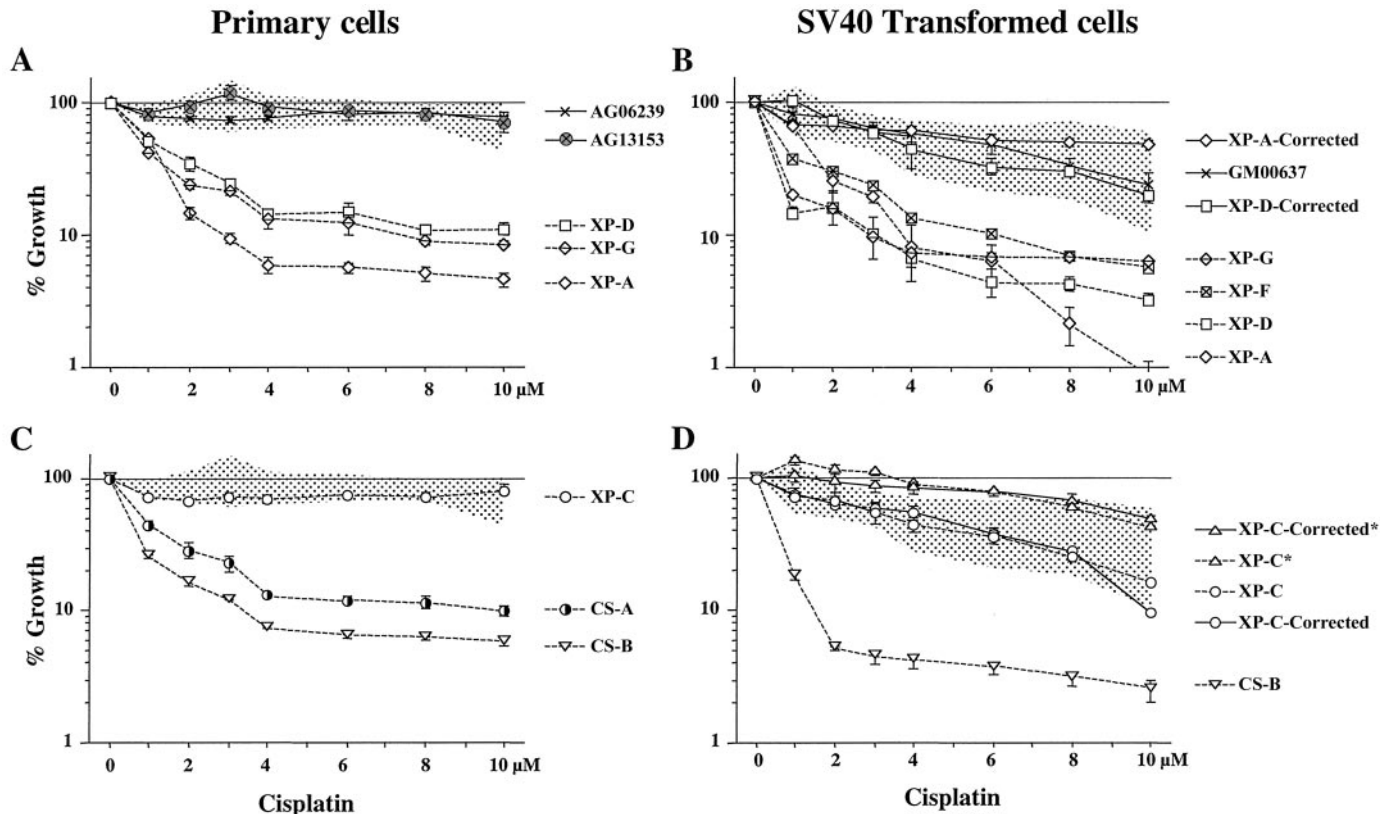
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Fig. 2. Differential antiproliferative activity of cisplatin in TC-NER- and GG-NER-deficient cells. *A*, primary cells with defects in the common NER pathway (*XP-D*, *XP-G*, and *XP-A*) are hypersensitive to cisplatin compared with normal primary fibroblasts (*AG06239* and *AG13153*). *B*, transformed cells with defects in the common NER pathway (*XP-G*, *XP-F*, *XP-D*, and *XP-A*) are hypersensitive to cisplatin compared with normal SV40-transformed fibroblasts (*GM00637*) and corrected cells (*XP-A-Corrected* and *XP-D-Corrected*). *C*, primary CS-A and CS-B cells are hypersensitive to cisplatin, whereas XP-C primary cells are resistant to cisplatin and exhibit similar response as normal cells (*panel A; shaded area*). *D*, SV40-transformed CS-B cells are hypersensitive to cisplatin, whereas SV40-transformed XP-C cells and XP-C cells from a patient tumor (*XP-C\**, XP44RO; *XP-C-corrected\**, XPC44RO+XPC) exhibit similar responses as normal cells irrespectively of correction (*shaded area*). *Bars*, SE. *Shaded areas*, ranges of sensitivity for primary or transformed cell lines proficient for both TC-NER and GG-NER.

DNA adducts. The XPC/hHR23B complex might not detect the cisplatin-DNA adduct, as suggested by the observation that the XPC/hHR23B complex has a low affinity for cisplatin adducts (17).

On the other hand, when one of the TC-NER-specific proteins, CSA or CSB, was deficient, we found that the cells were markedly hypersensitive to cisplatin. Interestingly, the $IC_{50}$s of CS-A and CS-B cells were almost the same as those of XP-A, XP-D, XP-F, and XP-G cells that are deficient for both TC-NER and GG-NER, which is consistent with the irrelevance of GG-NER for cisplatin antiproliferative activity. Moreover, transfection of the corresponding deficient gene in two of the TCR-deficient fibroblasts (*i.e.*, XP-A and XP-D) decreased the sensitivity to cisplatin to the levels of cells proficient for TC-NER including normal (NER proficient) and XP-C cells. These results indicate that a single deficiency in the TC-NER pathway leads to hypersensitivity to cisplatin, and that a defect of XPC in the GG-NER pathway has no effect on the cellular response to cisplatin.

Our multiple regression analysis also revealed that SV40 transformation, which blocks the function of p53 and pRb (18), enhanced cisplatin activity independently of the TC-NER status of the cells. Recent studies revealed that p53 deficiency sensitizes cells to both cisplatin and UV radiation (15). However, the mechanisms by which

p53 deficiency sensitizes to UV and cisplatin are likely to be different. In the case of UV, p53 enhances GG-NER without affecting TC-NER (19). In the case of cisplatin, the most plausible function of p53 in protecting cells against cisplatin is by activating the cell cycle checkpoint to extend the time for the repair of the DNA damage (19), probably not directly related to DNA repair, because we found that GG-NER does not contribute to the antiproliferative activity of cisplatin. However, cells with inactivation of p53 are sometimes resistant to cisplatin, presumably because p53-dependent apoptotic response is inactivated (3). Thus, we assume that the role of p53 in cisplatin response is complex and that p53 status cannot be used generally as a predictive marker of cellular response.

The response rates to cisplatin-based chemotherapy are consistently excellent in testicular tumors (16) but are unpredictable in ovarian cancers, for which more than half of the patients fail to respond either after the first course of chemotherapy or relapse (4). The availability of tumor markers would be most useful to predict which patients will benefit from cisplatin-based chemotherapy. A recent study suggested that the high response rate of testicular tumors was related to NER deficiencies in such tumors (16). Similarly, resistance of ovarian cancer to cisplatin is reported to be associated with increased NER activity (14). Because we found that loss of heterozygosity of NER factors is relatively frequent in ovarian cancer (20), differences in NER status among different tumors may underlie their sensitivity to cisplatin. Recently, the mechanism of action of Et743, a new anticancer drug in Phase II clinical trials, has been elucidated. In contrast to cisplatin, TC-NER-proficient cells are more sensitive to Et743 than

Table 2 *Multiple regression analysis of factors associated with cisplatin response*

|  | R | t | P |
|---|---|---|---|
| TC-NER | 12.3 | 3.6 | 0.002 |
| GG-NER | 2.3 | 0.7 | 0.5 |
| Primary/Transformed | 8.9 | 3.3 | 0.016 |

TC-NER-deficient cells (10), suggesting that cisplatin-resistant tumors should be considered a potential indication for treatment with Et743. However, in neither case was GG-NER determinant for drug responses. Together, these findings suggest that examination of the TC-NER status of cancer cells should be useful for the selection of cisplatin or Et743 in individual patients.

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