Featured Article

Sensitization to the Cytotoxicity of Cisplatin by Transfection with Nucleotide Excision Repair Gene Xeroderma Pigmentosun Group A Antisense RNA in Human Lung Adenocarcinoma Cells

Xiaoming Wu,[1] Wei Fan,[2] Shunqing Xu,[1] and Yikai Zhou[1]

Institute of Environmental Medicine[1] and Department of Immunology,[2] Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Peoples Republic of China

Abstract

Purpose: **The resistance of tumor cells to chemotherapeutic agents, such as cisplatin, remains an important problem to be solved in cancer chemotherapy. One of the mechanisms associated with cisplatin resistance is the enhanced nucleotide excision repair (NER) capacity. Because xeroderma pigmentosum group A (XPA) plays a central role at an early stage in the NER pathway, we are interested in whether down-regulation of *XPA* gene expression by antisense RNA transfection could reduce DNA repair and thus sensitize tumor cells to cisplatin.**

Experimental Design: **Human lung adenocarcinoma cells were stably transfected XPA antisense RNA expression vector, and six colonies were selected for determining the XPA mRNA level by Northern blot. The cell viability was measured by an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic assay, and the host cell reactivation was employed to assess the NER capacity of cisplatin-damaged luciferase reporter plasmid. Flow cytometry analysis was used to determine cisplatin-induced apoptosis.**

Results: **We showed that transfection with antisense XPA RNA could decrease the XPA mRNA level and sensitize tumor cells to cisplatin. This enhanced sensitivity can be attributed to the reduced NER capacity in transfected cells as measured by the host cell reactivation assay. Moreover, the XPA mRNA level is correlated significantly with both cisplatin IC_{50} value and cellular NER capacity. Furthermore, a more pronounced apoptotic response was observed in transfected cells treated by cisplatin.**

Conclusions: **Our results suggest that the targeted inhibition of XPA by antisense strategy may provide a valuable tool in clinical cancer chemotherapy.**

Introduction

NER[3] is a universal and versatile repair process capable of removing a large variety of DNA lesions from the genome, including UV-induced photoproducts, bulky chemical adduct, and certain types of DNA cross-links (1). The NER pathway consists of at least 30 proteins involved in sequential damage recognition, dual incision/excision, repair synthesis, and ligation steps (2), among which the XPA protein is a zinc-finger protein of M_r 31,000 (273 amino acids) involved in DNA damage recognition (3, 4). In GG-NER, binding of the XPA protein to the DNA lesion sites is an important early step of this repair pathway. The interaction of XPA with the M_r 34,000 subunit of replication protein A can activate XPA to recruit other NER components, such as the basal transcription factor TFIIH complex and ERCC1/XPF heterodimer, to the lesion site (1). In transcription-coupled NER, the coupling factor Cockayne Syndrome group B gene interacts with TFIIH and polymerase II, resulting in the recruitment of TFIIH to the stalled polymerase II at the lesion site. TFIIH then interacts with XPA and can bring XPA proximal to the lesion site (5–7). Because of the central role that XPA plays in DNA lesion recognition and its interaction with other NER repair proteins, XPA has been considered the damage-sensing and repair-recruitment factor of NER. Of all of the seven XP complementation groups (*i.e.*, A through G), cells from group A are the most severely impaired in NER and demonstrate an extreme *in vitro* sensitivity to UV and other types of bulky DNA damage (2).

Cisplatin [*cis*-diamminedichloroplatinum(II)] is a widely used chemotherapeutic agent and exerts its cytotoxic effects by disrupting the DNA structure in cells through the formation of, mainly, intrastrand adducts and interstrand cross-link (8). The repair of cisplatin-induced DNA damage is primarily performed by NER (9). The sensitivity of tumor cells to cisplatin has been reported to be inversely correlated with cellular NER capability (10–12). It has been shown that the overexpression of NER genes was associated with cisplatin resistance in human ovarian, glioma, bladder, and lung cancer cells (10, 13–15). Hence, we hypothesize that down-regulation of NER genes expression re-

Received 1/22/03; revised 5/12/03; accepted 5/31/03.

Grant support: National Natural Science Foundation of China (No. 39990570).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Yikai Zhou, Institute of Environmental Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Peoples Republic of China. Phone: 86-27-83692703; Fax: 86-27-83692701; E-mail: zhouyk@mails.tjmu.edu.cn.

[3] The abbreviations used are: NER, nucleotide excision repair; TFIIH, trancription factor IIH; ERCC1, excision repair cross-complementing rodent repair deficiency, complementation group 1; XPA, xeroderma pigmentosun group A; HCR, host cell reactivation; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; β-gal, β-galactosidase; GG, global genome.

duces DNA repair and, thus, sensitizes tumor cells to anticancer agents.

Because XPA has a crucial role at an early stage of both GG-NER and transcription-coupled NER, the protein is an excellent target for gene therapeutic intervention through sensitization of tumor cells to chemotherapy (16). We stably transfected an antisense construct of XPA into human lung adenocarcinoma cells proficient in NER to determine whether the inhibition of the *XPA* gene would enhance the sensitivity of the cells to cisplatin. We found that XPA antisense RNA could reduce cellular DNA repair capacity and sensitize the cells to cisplatin.

Materials and Methods

Cell Culture. We used a human lung adenocarcinoma cell line A549 (purchased from Wuhan University, Wuhan, China) in all of the experiments. The cells have normal NER and p53 functions (16, 17) and were routinely maintained in DMEM containing 10% fetal bovine serum (Life Technologies, Inc.) supplemented with penicillin and streptomycin. We selected the clones (designated as AS1 to AS6) from transfected A549 cells with the XPA antisense expression plasmid and kept them growing in DMEM with 250 μg/ml G418.

Plasmid Constructs. We isolated total RNA from the cancer cells using Trizol reagent (Life Technologies, Inc.) and performed reverse transcription by using oligo$(dT)_{15}$ primer and Moloney murine leukemia virus reverse transcriptase (Promega Biotech). A 426-bp 5′ end of the reverse-transcribed XPA cDNA was amplified by PCR. The 5′-sense (forward) primer used was: 5′-TAA*TCTAGA*GCTAGGTCCTCGGAGTGG-3′. The antisense primer (reverse) was: 5′-CGG*GAATTC*TCATCAGCATCTCTGCAGT-3′, by which we introduced the *Xba*I and *Eco*RI restriction sites (shown by italics), respectively. The final volume of reaction was 50 μl, containing 1 μl of cDNA mixture, 1× PCR buffer, 0.1 mM each dNTP, 2 units of Taq polymerase, 10 pmol of each *XPA* gene primer. The PCR amplification was performed for 35 cycles as follows: at 94°C for 30 s, at 69°C for 30 s, and at 72°C for 45 s, with a final extension at 72°C for 7 min. The PCR product was digested and inserted in the inverted orientation into *Eco*RI and *Xba*I sites of the pcDNA3.1 vector (Invitrogen, Carlsbad, CA). The identity of the insert was confirmed by DNA sequencing and compared with human *XPA* gene (Genbank accession no. NM-000380).

Transfection. A549 cells, grown to 50–80% confluence, were transfected with purified XPA antisense expression plasmid using LipofectAMINE reagent according to the manufacturer's instructions (Invitrogen, Life Technologies). Meanwhile, cells were also transfected in parallel with an empty vector to serve as a mock transfection control. The transfected clones were selected using limiting dilution when the G418 (800 μg/ml)-resistant colonies appeared. The selection was performed by determining the levels of XPA mRNA expression in cell colonies by Northern blotting.

Northern Blot Analysis. The expression of XPA mRNA in A549 cells and six transfected clones (AS1 to AS6) was determined by using DIG High Prime DNA Labeling and Detection Starter Kit II, as suggested by the manufacturer (Roche Diagnostics). Briefly, RNA was prepared using the Trizol reagent, separated on 1% formaldehyde-agarose gel (approximately 30 μg of each RNA sample), blotted onto nylon membrane by capillary transfer, and hybridized with DIG-labeled strand-specific DNA probes. After overnight hybridization and stringency washes, the membrane was incubated with Anti-Digoxigenin-AP(1:10,000) and was visualized by exposure to X-ray film.

Cytotoxicity Assay. We used a MTT assay to determine the cell viability as an indicator for the relative sensitivity of the cells to cisplatin and Adriamycin. Cells growing in the logarithmic phase were seeded in 96-well plates (1×10^4/well), allowed to attach overnight, and then were treated with varying doses of cisplatin and Adriamycin (Sigma) for 24 h. In time-course experiments, the treated cells were washed with PBS and were further incubated for varying posttreatment periods (*i.e.*, 24 h, 48 h, and 72 h). Aliquots of 10 μl of MTT (5 mg/ml) were then added to each well. After 4 h, the color formed was quantitated by a spectrophotometric plate reader (BIO-TEK INSTRUMENTS, INC) at 490 nm wavelength after solubilization in 150 μl of DMSO.

HCR Assay. HCR of luciferase activity was determined as described previously with modification (18). Briefly, the reporter plasmid pGL3 (Promega Biotech, Madison, WI) containing the luciferase gene driven by the SV40 promoter was treated for 12 h at 37°C with increasing concentrations (*i.e.*, 40,200 and 1,000 ng/ml) of cisplatin in TE buffer [10 mM Tris (pH 8.0)—1 mM EDTA] at a DNA concentration of 200 μg/ml. The damaged plasmid was recovered by precipitation with ethanol, and after it was dissolved in TE buffer, the concentration of plasmid was determined. The parental A549 cells and transfected clones were seeded in 24-well plates and were grown to 50–80% confluence. The cells were transiently transfected with 1 μg of cisplatin-damaged reporter plasmid and 1 μg of control plasmid (pSV-β-gal; Promega) by LipofectAMINE reagent according to the manufacturer's instructions. Cells were collected 48 h after transfection, and cell extracts were used to determine luciferase activity (Luciferase Assay System with Reporter Lysis Buffer; Promega) and β-gal activity. Luciferase activity values were quantified with a luminometer (Lumat, LB9507; EG&G) and were normalized for β-gal activity.

Apoptosis Assay. Apoptotic cells, defined as those with less than the G_0-G_1 DNA content, were measured according to the protocol described previously (19). Briefly, the adherent and floating cells were harvested by centrifugation at 400 × *g* for 5 min, and were fixed in 1 ml of ice-cold 70% ethanol at 4°C overnight. The cells were centrifuged and resuspended in 0.5 ml of staining solution (50 μg of propidium iodide per ml, 100 units per ml RNase in PBS) and were incubated at 37°C for 30 min. The samples were then analyzed on a FACScan flow cytometry (Becton Dickinson).

Results

Down-Regulation of XPA mRNA Level by Antisense RNA Transfection. Human lung adenocarcinoma cells A549 were stably transfected with the recombinant plasmid containing 426-bp 5′ end of the human XPA cDNA in the antisense orientation. Six antisense G-418 resistant colonies were selected and determined for XPA mRNA expression (Fig. 1). The transfected cells expressed various levels of XPA mRNA, and the

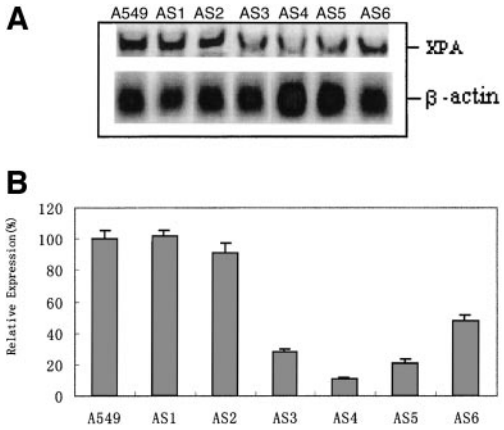

Fig. 1 Down-regulation of XPA mRNA level by antisense RNA transfection. *A*, the expression of XPA mRNA in the parental A549 cells and stably transfected colonies (AS1–AS6) was assessed by Northern blotting, as described in "Materials and Methods." The results are representative of three independent experiments. *B*, the relative XPA mRNA level was quantified after normalization to β-actin expression (mean ± SD).

decreased expression was observed in AS3–AS6 colonies, but not in AS1–AS2 colonies and control cells. Also, the AS3–AS6 colonies displayed a significant decrease in XPA protein level as revealed by Western blot (data not shown). We did not observe any morphological or growth rate differences between the antisense-transfected cells and the parental cells.

Transfection of Antisense RNA Increases Cisplatin Toxicity. To determine whether decreased expression of XPA mRNA enhanced cisplatin sensitivity, the cells were exposed to increasing doses of cisplatin for 24 h, and cell viability was measured by the MTT assay. As shown in Fig. 2*A*, the transfected cells with decreased XPA mRNA level (AS3–AS6) exhibited increased sensitivity to cisplatin, whereas colonies of AS1 and AS2 were about as sensitive as the parental cells. The decreased XPA mRNA levels were found to be associated with increased cisplatin sensitivity. The statistical analysis revealed that XPA mRNA levels correlated significantly with the cisplatin IC_{50} value (Fig. 2*A*). In time-course experiments, the cells were treat with 1 μg/ml cisplatin for 24 h, and were further incubated for 24–72 h. The transfected cells with decreased XPA mRNA level showed a significantly greater reduction in cell viability over time, compared with the parental cells (Fig. 2*B*). Interestingly, when the assay was applied to another clinically important drug, Adriamycin, the anticancer action of Adriamycin was attributed to the production of DNA double-strand breaks (20–22). We found no significant cytotoxicity difference in the transfected and parental cells (Fig. 3).

Transfection with Antisense RNA Reduces Repair of Cisplatin-Damaged Plasmid. To investigate whether the enhanced sensitivity to cisplatin in cells expressing decreased levels of XPA mRNA was due to reduced DNA repair capacity, a host-cell reactivation assay was conducted in which cisplatin-damaged reporter plasmid was transiently transfected into cells, and the greater the relative reporter gene activity, the more proficient the DNA repair of host cells. As shown in Fig. 4, the inhibition of XPA expression by antisense RNA, indeed, led to a significant decrease in reactivation of the reporter plasmid damaged by various doses of cisplatin. A statistically significant correlation was observed between the relative expression of XPA mRNA and luciferase activity. These results indicated that decreased DNA repair capacity in colonies of AS3–AS6 contributed to, at least in part, the enhanced sensitivity to cisplatin in these cells.

Enhanced Apoptosis in Cells with Reduced Level of XPA mRNA after Cisplatin Treatment. One important way by which cisplatin kill cells is through the induction of apoptosis (23). We were interested in whether increased cisplatin cytotoxicity in cells with a reduced level of XPA mRNA was the result of enhanced apoptosis. After 24 h of cisplatin treatment, both the detached and the attached cells were collected for the flow cytometry assay. At the doses of 5 and 20 μg/ml, cisplatin induced 7.4% and 14.5%, respectively, of the parental A549 and

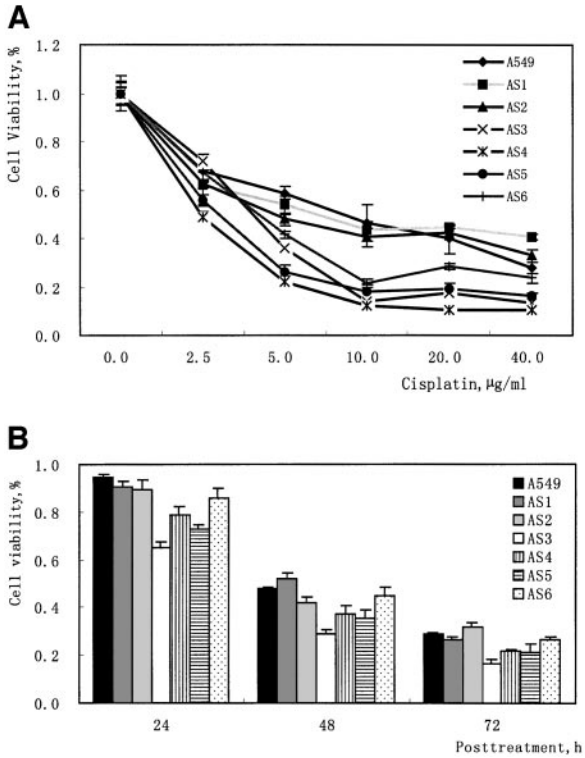

Fig. 2 The effect of transfection with XPA antisense RNA on the cellular cisplatin sensitivity. *A*, the cells were treated with various doses of cisplatin for 24 h, and the cell viability was measured by a MTT assay. Each data point represents the mean for four wells (mean ± SD). The colonies of the transfected AS3–AS6 cells exhibited significantly increased sensitivity to cisplatin compared with the parental cells (SAS software package Version 8.0; ANOVA analysis: F = 9.75, 9.14, 7.39, 8.91; P = 0.005, 0.006, 0.012, 0.006, respectively), whereas this increased sensitivity was not observed in colonies of AS1 and AS2 (F = 0.43, 2.44; P = 0.517, 0.131, respectively). The relationship of XPA mRNA level with the cisplatin cytotoxicity (IC_{50} value) was analyzed by the univariate linear regression analysis and revealed a significant correlation (r = 0.927; P = 0.003). *B*, the cells were treated with 1 μg/ml cisplatin for 24 h, were washed with PBS, and were further incubated for 24–72 h; then the cell viability was determined by MTT assay.

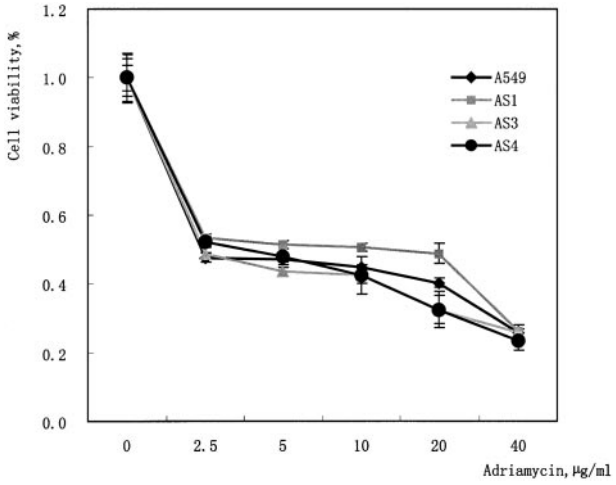

Fig. 3 Transfection with XPA antisense RNA has no effect on cellular adriamycin sensitivity. The cells were treated with various doses of Adriamycin for 24 h, and the cell viability was measured by a MTT assay. Each data point represents the mean for four wells (mean ± SD). There are no statistically significant differences in cell viability between the parental cells and transfected cells (AS1, AS3, and AS4; $P > 0.05$). The experiment was carried out three times with similar results.

21.1% and 55.7% of the transfected AS4 cells to undergo apoptosis. In the meantime, the colonies of AS4 exhibited more apparently fragmented DNA after cisplatin treatment (data not shown). Similarly, the colonies of AS3, AS5, and AS6 also demonstrated the enhanced apoptosis by cisplatin treatment (data not shown).

Discussion

Cisplatin is widely used for the treatment of many malignancies, including testicular, ovarian, bladder, cervical, head and neck, and small-cell and non-small-cell lung cancer. The cytotoxicity of this drug is believed to be the result of the formation of platinum-DNA adducts. Intracellularly, cisplatin becomes hydrated to form an electrophilic species that binds preferentially to the N7 atom of guanine and adenine residues (24). Platinum–DNA adducts comprise primarily monoadducts and intrastrand cross-links and a small percentage of GG-interstrand cross-links and DNA-protein cross-links (25–27). Despite the great efficacy in treating certain kinds of cancers, the use of cisplatin in cancer chemotherapy is limited by acquired or intrinsic resistance to the drug in treated cells. Previous studies indicated that cisplatin resistance is multifactorial, consisting of mechanisms such as impaired cellular drug uptake, increased drug inactivation, increased tolerance of platinum–DNA damage, and enhanced repair of DNA damage (15, 27–30). Accordingly, it is expected that modulation of these factors might be able to overcome the cisplatin resistance, thereby enhancing the therapeutic potential of the drug.

Several families of proteins that can recognize cisplatin-induced DNA damage have been suggested involved in cisplatin resistance, including NER proteins, mismatch repair proteins (31), DNA-dependent protein kinase (DNA-PK; Ref. 32), and high-mobility group proteins (33). NER is believed to be the main pathway responsible for the repair of cisplatin–DNA adducts. Enhanced NER has been found to be associated with increased resistance to cisplatin in human ovarian and lung cancer cells (30, 34–35). Particularly, increased expression of XPA mRNA and other NER genes such as *ERCC1*, *XPB*, and *XPC* was observed in the cancer cells that are resistant to cisplatin chemotherapy (10, 36–37). On the other hand, mammalian cells deficient in NER are more sensitive to cisplatin than are the corresponding wild-type cells. Restoration of NER with purified protein increased the survival of repair-deficient cells. Of great importance is the recent observation that testicular tumors cells with low levels of the XPA protein and the ERCC1-XPF endonuclease complex are sensitive to many different chemotherapeutic drugs including cisplatin (38).

In this report, we demonstrated that down-regulation of XPA mRNA by antisense strategy inhibited NER capacity, and, thus, enhanced the sensitivity to cisplatin in a human lung cancer cell line. Moreover, a significant correlation was found between the XPA mRNA expression with the cisplatin cytotoxicity and NER capacity (Figs. 2*A* and 4). Although such correlation was not found by Damia *et al.* (39), a similar study by Yang *et al.* (40) did observe a correlation between the level of ERCC1 mRNA with the *in vitro* NER capacity. Our observations suggested that the level of XPA mRNA is also an important determinant in the NER pathway (41), consistent with the report that the amount of XPA protein expressed in fibroblasts is a limiting factor for NER capacity (42). Nevertheless, the sensitivity to Adriamycin is only slightly influenced in antisense-transfected cells (Fig. 3), and the cellular resistance to Adriamycin has been linked with mismatch repair and DNA double-strand break repair (43, 44). Our data further suggest that the increase in cisplatin sensitivity could be attributed to the reduced NER capacity as a result of a reduced level of XPA mRNA expression.

One important mechanism of translation of cisplatin–DNA

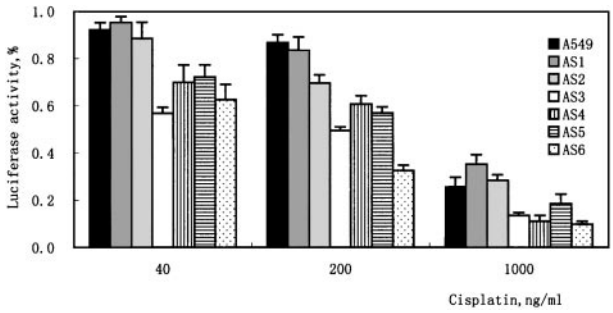

Fig. 4 Transfection with XPA antisense RNA reduces HCR of cisplatin-damaged luciferase reporter plasmid. The reporter plasmid pGL3 was treated with 40, 200, and 1000 ng/ml cisplatin for 12 h, respectively, and was recovered by precipitation with ethanol. The cells were transiently transfected with 1 μg of cisplatin-damaged reporter plasmid and 1 μg of control plasmid (pSV-β-gal). At 48 h after transfection, cells were harvested; then luciferase and β-gal activity were analyzed as described in "Materials and Methods." A statistically significant correlation was observed between XPA mRNA level with the HCR capacity of cisplatin-damaged reporter plasmid ($r = 0.854, 0.696, 0.858$; $P = 0.014, 0.082$, and 0.013, corresponding to 40, 200, and 1000 ng/ml cisplatin treatment, respectively).

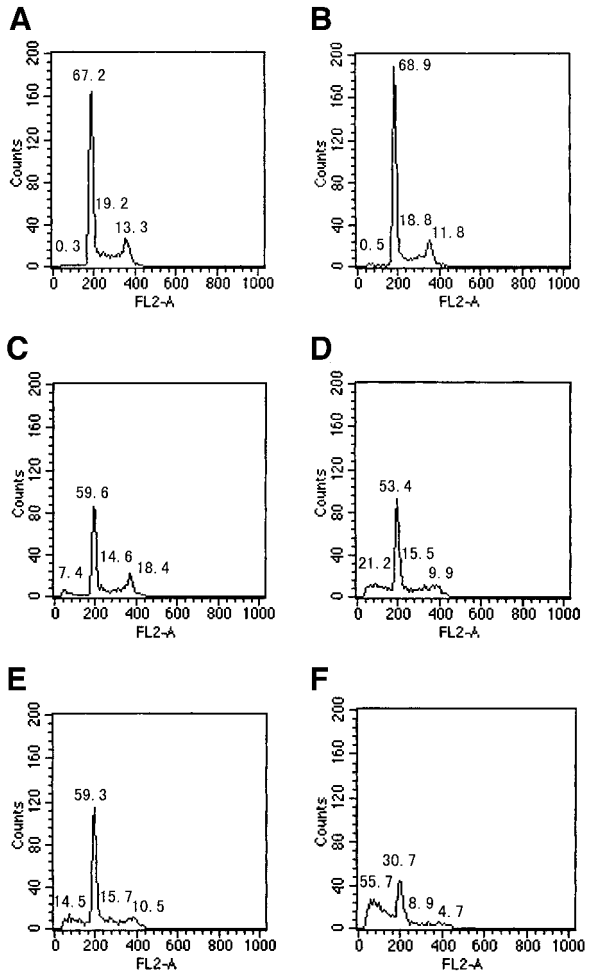

Fig. 5 Treatment with cisplatin induces enhanced apoptosis in antisense-transfected cells. The parental A549 cells and colonies of AS4 were treated with 5 and 20 μg/ml cisplatin for 24 h; then the apoptotic cells were measured as described in "Materials and Methods." *A*, control-parental A549 cells; *B*, control-AS4 cells; *C*, treatment with 5 μg/ml cisplatin-parental A549 cells; *D*, treatment with 5 μg/ml cisplatin-AS4 cells; *E*, treatment with 20 μg/ml cisplatin-parental A549 cells; *F*, treatment with 20 μg/ml cisplatin-AS4 cells. FL2-A, fluorescence light-2 area.

damage into cell death is apoptosis. Emerging evidence has suggested that a large number of cancer patients may have developed cisplatin resistance as the result of a reduced apoptotic response (23). In this case, increased levels of DNA damage would be required to induce the signal that initiates apoptosis. The results from this study demonstrated that the inefficient repair of the DNA damage induced by cisplatin in the antisense-transfected cells could lead to enhanced apoptosis (Fig. 5). Again, we observed a more pronounced activation of caspase-3 in the transfected cells by cisplatin treatment (data not shown).

In summary, our results suggest that the specific inhibition of XPA mRNA by antisense strategy could decrease NER capacity and sensitize human lung adenocarcinoma cells to cisplatin and might be able to sensitize other types of tumor cells to other drugs known to cause bulky lesions repaired by NER. XPA is a unique potential target for chemotherapy among the core NER factors because it has no known involvement in other cellular pathways. This apparently unique role of XPA is in sharp contrast to the components replication protein A, TFIIH, XPG, and ERCC1-XPF, each of which has some other function in a separate process of DNA replication, transcription, or recombination, or in another repair pathway (38, 45–47). Therefore, targeted inhibition of XPA might be a valuable tool in clinical cancer chemotherapy.

Acknowledgments

We are highly thankful to Dr. Qinyi Wei (Anderson Cancer Center, University of Texas) for critical reading of the manuscript and helpful suggestions.

References

1. Wood, R. D. DNA repair in eukaryotes. Annu. Rev. Biochem., *65:* 135–167, 1996.

2. de Laat, W. L., Jaspers, N. G. J., and Hoeijmakers, J. H. J. Molecular mechanism of nucleotide excision repair. Genes Dev., *13:* 768–785, 1999.

3. Tanaka, K., Miura, N., Satokata, I., Miyamoto, I., Yoshida, M. C., Satoh, Y., Kondo, S., Yasui, A., Okayama, H., and Okada, Y. Analysis of human excision repair gene involved in group A xeroderma pigmentosum and containing a zinc-finger domain. Nature (Lond.), *348:* 73–76, 1990.

4. Jones, C. J., and Wood, R. D. Preferential binding of the xeroderma pigmentosum A complementing protein to damaged DNA. Biochemistry, *32:* 12096–12104, 1993.

5. Tantin, D., Kansal, A., and Carey, M. Recruitment of putative transcription-repair coupling factor CSB/ERCC6 to RNA polymerase II elongation complexes. Mol. Cell. Biol., *17:* 6803–6814, 1997.

6. Tantin, D. RNA polymerase II elongation complexes containing the Cockayne syndrome group B protein interact with a molecular complex containing the transcription factor IIH components xeroderma pigmentosum B and p62. J. Biol. Chem., *273:* 27794–27799, 1998.

7. Selby, C. P., and Sancar, A. Human transcription-repair coupling factor CSB/ERCC6 is a DNA stimulated ATPase but is not a helicase and dose not disrupt the ternary transcription complex of stalled RNA polymerase II. J. Biol. Chem., *272:* 1885–1890, 1997.

8. Johnes, J., Zhen, W., Reed, E., Parker, R. J., Sancar, A., and Bohr, V. A. Gene-specific formation and repair of cisplatin intrastrand adducts and interstrand cross-links in Chinese hamster ovary. J. Biol. Chem., *266:* 7101–7107, 1991.

9. Sancar, A. Excision repair in mammalian cells. J. Biol. Chem., *270:* 15915–15918, 1995.

10. Ferry, K. V., Hamilton, T. C., and Johnson, S. W. Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: role of ERCC1-XPF. Biochem. Pharmacol., *60:* 1305–1313, 2000.

11. Furuta, T., Ueda, T., Aune, G., Sarasin, A., Kraemer, K. H., and Pommier, Y. Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. Cancer Res., *62:* 4899–4902, 2002.

12. Damia, G., Imperatori, L., Stefanini, M., and D'Incalci, M. Sensitivity of CHO mutant cell lines with specific defects in nucleotide excision repair to different anti-cancer agents. Int. J. Cancer, *66:* 779–783, 1996.

13. Aloyz, R., Xu, Z. Y., Bello, V., Bergeron, J., Han, F. Y., Yan, Y., Malapetsa, A., Alaoui-Jamali, M. A., Duncan, A. M., and Panasci, L. Regulation of cisplatin resistance and homologous recombinational repair by the TFIIH subunit XPD. Cancer Res., *62:* 5457–5462, 2002.

14. Xu, Z., Chen, Z. P., Malapetsa, A., Alaoui-Jamali, M., Bergeron, J., Monks, A., Myers, T. G., Mohr, G., Sausville, E. A., Scudiero, D. A.,

Aloyz, R., and Panasci, L. C. DNA repair protein levels vis-a-vis anticancer drug resistance in the human tumor cell lines of the National Cancer Institute drug screening program. Anticancer Drugs, *13:* 511–519, 2002.

15. Kartalou, M., and Essigmann, J. M. Mechanisms of resistance to cisplatin. Mutat. Res., *478:* 23–43, 2001.

16. Rosenberg, E., Taher, M. M., Kuemmerle, N. B., Farnsworth, J., and Valerie, K. A truncated human xeroderma pigmentosum complementation group A protein expressed from an adenovirus sensitizes human tumor cells to ultraviolet light and cisplatin. Cancer Res., *61:* 764–770, 2001.

17. O'Connor, P. M., Jackman, J., Bae, I., Myers, T. G., Fan, S., Mutoh, M., Scudiero, D. A., Sausville, E. A., Weinstein, J. N., Friend, S., Fornace, A. J., and Kohn, K. W. Characterization of the p53 tumor suppressor pathway in cell lines of the National Cancer Institute anticancer drug screen and correlations with the growth-inhibitory potency of 123 anticancer agents. Cancer Res., *57:* 4285–4300, 1997.

18. Fan, J., and Bertino, J. R. Modulation of cisplatinum cytotoxicity by p53: effect of p53-mediated apoptosis and DNA repair. Mol. Pharmacol., *56:* 966–972, 1999.

19. Nicoletti, I., Migliorati, G., Pagliacci, M. C., Grignani, R., and Riccardi, C. A rapid and single method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J. Immunol. Methods, *139:* 271–279, 1991.

20. Tewey, K. M., Chen, G. L., Nelson, E. M., and Liu, L. F. Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J. Biol. Chem., *259:* 9182–9187, 1984.

21. Cummings, J., Anderson, L., Willmott, N., and Smyth, J. F. The molecular pharmacology of doxorubicin *in vivo*. Eur. J. Cancer, *27:* 532–535, 1991.

22. Skladanowski, A., and Konopa, J. Relevance of interstrand DNA crosslinking induced by anthracyclines for their biological activity. Biochem. Pharmacol., *47:* 2279–2287, 1994.

23. Gonzalez, V. M., Fuertes, M. A., Alonso, C., and Perez, J. M. Is cisplatin-induced cell death always produced by apoptosis? Mol. Pharmacol., *59:* 657–663, 2001.

24. Eastman, A. The formation, isolation and characterization of DNA adducts produced by anticancer platinum complexes. Pharmacol. Ther., *34:* 155–166, 1987.

25. Plooy, A. C., Fichtinger-Schepman, A. M., Schutte, H. H., van Dijk, M., and Lohman, P. H. The quantitative detection of various Pt-DNA-adducts in Chinese hamster ovary cells treated with cisplatin: application of immunochemical technique. Carcinogenesis (Lond.), *6:* 561–566, 1985.

26. Fichtinger-Schepman, A. M., van der Veer, J. L., den Hartog, J. H., Lohman, P. H., and Reedijk, J. Adducts of the antitumor drug *cis*-diamminedichloroplatinum(II) with DNA: formation, identification, and quantitation. Biochemistry, *24:* 707–713, 1985.

27. Lelland, L. R., Mistry, P., Abel, G., Loh, S. Y., O'Neill, C. F., Murrer, B. A., and Harrap, K. R. Mechanism-related circumvention of acquired *cis*-diamminedichloroplatinum(II) resistance using two pairs of human ovarian carcinoma cell lines by ammine/amine platinum(IV) dicarboxylates. Cancer Res., *52:* 3857–3864, 1992.

28. Godwin, A. K., Meister, A., O'Dwyer, P. J., Huang, C. S., Hamilton, T. C., and Anderson, M. E. High resistance to cisplatin in human ovarian cancer cell lines is associated marked increase of glutathione synthesis. Proc. Natl. Acad. Sci. USA, *89:* 3070–3074, 1992.

29. Johnson, S. W., Laub, P. B., Beesley, J. S., Ozols, R. F., and Hamilton, T. C. Increased platinum-DNA damage tolerance is associated with cisplatin resistance and cross-resistance to various chemotherapeutic agents in unrelated human ovarian cancer cell lines. Cancer Res., *57:* 850–856, 1997.

30. Zhen, W., Link, C. J. Jr., O'Connor, P. M., Reed, E., Parker, R., Howell, S. B., and Bohr, V. A. Increased gene-specific repair of cisplatin interstrand cross-link in cisplatin-resistant human ovarian cancer cell lines. Mol. Cell. Biol., *12:* 3689–3698, 1992.

31. Aebi, S., Kurdi-Haidar, B., Gordon, R., Cenni, B., Zheng, H., Fink, D., Christen, R. D., Boland, C. R., Koi, M., Fishel, R., and Howell, S. B. Loss of DNA mismatch repair in acquired resistance to cisplatin. Cancer Res., *56:* 3087–3090, 1996.

32. Turchi, J. J., and Henkels, K. Human KU antoantigen binds cisplatin-damaged DNA but fails to stimulate human DNA-activated protein kinases. J. Biol. Chem., *271:* 13861–13867, 1996.

33. Pil, P. M., and Lippard, S. J. Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin. Science (Wash. DC), *256:* 234–237, 1992.

34. Zeng, R. N., Paterson, J., Alpert, L., Tsao, M. S., Viallet, J., and Alaoui-Jamali, M. A. Elevated DNA repair capacity is associated with intrinsic resistance of lung cancer to chemotherapy. Cancer Res., *55:* 4760–4764, 1995.

35. Shellard, S. A., Fichtinger-Schepman, A. M. J., Lazo, J. S., and Hill, B. T. Evidence of differential cisplatin-DNA adduct formation, removal and tolerance of DNA damage in three human lung carcinoma cell lines. Anticancer Drugs, *4:* 491–500, 1993.

36. Dabholkar, M., Vionnet, J., Bostick-Bruton, F., Yu, J. J., and Reed, E. Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. J. Clin. Investig., *94:* 703–708, 1994.

37. Dabholkar, M., Bostick-Bruston, F., Weber, C., Bohr, V. A., Egwuagu, C., and Reed, E. ERCC1 and ERCC2 expression in malignant tissues from ovarian cancer patients. J. Natl. Cancer Inst. (Bethesda), *84:* 1512–1517, 1992.

38. Koberle, B., Masters, J. R., Hartley, J. A., and Wood, R. D. Defective repair of cisplatin-induced DNA damage caused by reduced XPA protein in testicular germ cell tumors. Curr. Biol., *9:* 273–276, 1999.

39. Damia, G., Guidi, G., and D'Incalci, M. Expression of genes involved in nucleotide excision repair and sensitivity to cisplatin and melphalan in human cancer cell lines. Eur. J. Cancer, *34:* 1783–1788, 1998.

40. Yang, L., Li, L., Jiang, H., Shen, Y., and Plunkett, W. Expression of ERCC1 antisense RNA abrogates gemcitabine-mediated cytotoxic synergism with cisplatin in human colon tumor cells defective in mismatch repair but proficient in nucleotide excision repair. Clin. Cancer Res., *6:* 773–781, 2000.

41. Muotri, A. R., Marchetto, M. C., Suzuki, M. F., Okazaki, K., Lotfi, C. F., Brumatti, G., Amarante-Mendes, G. P., and Menck, C. F. Low amounts of the DNA repair XPA protein are sufficient to recover UV-resistance. Carcinogenesis (Lond.), *23:* 1039–1046, 2002.

42. Cleaver, J. E., Charles, W. C., McDowell, M. L., Sadinski, W. J., and Mitchell, D. L. Overexpression of the *XPA* repair gene increase resistance to ultraviolet-radiation in human-cells by selective repair of DNA damage. Cancer Res., *55:* 6152–6160, 1995.

43. Shen, H., Schultz, M., Kruh, G. D., and Tew, K. D. Increased expression of DNA-dependent protein kinase confers resistance to Adriamycin. Biochim. Biophys. Acta, *1381:* 131–138, 1998.

44. Drummond, J. T., Anthoney, A., Brown, R., and Modrich, P. Cisplatin and Adriamycin resistance are associated with MutL α and mismatch repair deficiency in an ovarian tumor cell line. J. Biol. Chem., *271:* 19645–19648, 1996.

45. Wilson, D. M., and Thompson, L. H. Life without DNA-repair. Proc. Natl. Acad. Sci. USA, *94:* 12754–12757, 1997.

46. Klungland, A., Hoss, M., Gunz, D., Constantinou, A., Clarkson, S. G., and Doetsch, P. W. Base excision-repair of oxidative DNA damage activated by XPG protein. Mol. Cell, *3:* 33–42, 1999.

47. Wang, X. W., Vermeulen, W., Coursen, J. D., Gibson, M., Lupold, S. E., Forrester, K., Xu, G., Elmore, L., Yeh, H., Hoeijmakers, J. H. J., and Harris, C. C. The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. Genes Dev., *10:* 1219–1232, 1996.