PERGAMON

European Journal of Cancer 36 (2000) 1869–1875

European Journal of Cancer

www.ejconline.com

Mechanism of the combination effect of wild-type *TP53* gene transfection and cisplatin treatment for ovarian cancer xenografts

M. Shimada *, J. Kigawa, Y. Kanamori, H. Itamochi, M. Takahashi, S. Kamazawa, S. Sato, N. Terakawa

Department of Obstetrics and Gynecology, Tottori University School of Medicine, 36-1 Nishimachi, Yonago 6838504, Japan

Received 24 January 2000; received in revised form 15 March 2000; accepted 23 March 2000

Abstract

To clarify the effect of a combination treatment consisting of a recombinant adenovirus carrying a wild-type *TP53* gene (AxCA*TP53*) and cisplatin (CDDP), we examined p53-dependent apoptosis in ovarian cancer xenografts with and without the wild-type *TP53* gene. Severe combined immunodeficiency (SCID) mice were implanted with ovarian cancer cell lines consisting of SK-OV-3 cells without the *TP53* gene and KF cells with the *TP53* gene. In SK-OV-3 and KF tumours, the inhibitory effect of the combination treatment on tumour growth was significant, compared with a single treatment with CDDP alone or AxCA*TP53* alone. The apoptotic index increased significantly after combination treatment in the SK-OV-3 tumours. The expression of Bax protein in SK-OV-3 tumours was weak, but strengthened after *TP53* gene transfection. In contrast, AxCA*TP53* transfection did not affect CDDP-induced apoptosis in the KF tumours. Therefore, combination treatment of AxCA*TP53* and CDDP may be a new strategy for treating ovarian cancer with or without the *TP53* gene. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Ovarian cancer; CDDP; *TP53* gene transfer; Recombinant adenovirus; Xenograft

1. Introduction

Epithelial ovarian cancer, one of the major causes of cancer death in women, is usually not diagnosed until it is an advanced stage [1,2]. Cytoreductive surgery followed by platinum-based combination chemotherapy is currently the standard treatment of ovarian cancer. Cisplatin (CDDP), a key drug in chemotherapy for ovarian cancer, has achieved a high response rate. However, the development of resistance to CDDP limits treatment success [3,4]. As a result, the survival rate for patients with epithelial ovarian cancer has not improved over the past 20 years [5]. Therefore, a new treatment approach is needed to improve the prognosis of patients with ovarian cancer.

Recently, several studies showed *TP53* gene transfer may be potentially useful in cancer gene therapy [6–9]. We also developed a new recombinant adenovirus carrying a wild-type *TP53* gene (AxCA*TP53*) and demonstrated that AxCA*TP53* transfection increased the sensitivity of SK-OV-3 cells to CDDP. These cells harbour a deletion of the *TP53* gene [10]. The combination of *TP53* gene transfection and CDDP may be an effective strategy for treating CDDP-resistant ovarian cancer. However, the mechanism of synergy in tumour suppression with this combination therapy has not been defined. Although a study of lung cancer cells indicated that the sequence of CDDP administration and *TP53* gene transfer is critical for a combination effect [11], the effectiveness of differing sequential administrations of the *TP53* gene and CDDP is presently unclear for *in vivo* ovarian cancer.

p53 is a transcriptional regulator of the cellular response to DNA damage [12]. Nuclear accumulation of p53 is enhanced by DNA-damaging agents such as CDDP [13,14]. p53 can function not only in the induction of apoptosis, but also in DNA repair. However, whether and how *TP53* gene transfection affects the sensitivity of cells to CDDP with wild-type *TP53* is unknown.

The present study was conducted to determine a useful sequential combination therapy of AxCA*TP53* and

* Corresponding author. Tel.: +81-859-34-8127; fax: +81-859-34-8089.

E-mail address: kigawa@grape.med.tottori-u.ac.jp (M. Shimada).

PII: S0959-8049(00)00161-1

CDDP and to examine p53-dependent apoptosis in ovarian cancer xenografts with and without wild-type *TP53*.

2. Materials and methods

This study was performed at the Laboratory Animal Research Center, under the control of the animal research committee in accordance with the Guidelines for Animal Experimentation in the Faculty of Medicine, Tottori University, Yonago, Japan.

A human ovarian adenocarcinoma cell line with a homozygous deletion of the *TP53* gene, SK-OV-3, was obtained from the American Type Culture Collection and a human ovarian adenocarcinoma cell line with wild-type *TP53*, KF, was kindly provided by Dr Kikuchi, National Defense Medical College (Tokorozawa, Japan). SK-OV-3 cells were maintained in minimum essential medium (Nissui, Tokyo, Japan) with 10% fetal calf serum (FCS) in a humidified atmosphere containing 5% CO_2 at 37°C. KF tumours were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui, Tokyo, Japan) with 10% FCS also in 5% CO_2 at 37°C.

For *TP53* gene transfection, we used a recombinant adenovirus carrying wild-type *TP53* (AxCA*TP53*). A recombinant adenovirus AxCA*LacZ*, encoding for the bacterial *LacZ* gene, was used as a reporter gene. These recombinant adenoviruses can replicate only in the 293 cells including the Ad5 E1 region in chromosomal DNA. The recombinant adenoviruses were grown and propagated in the 293 cells, and maintained in Dulbecco's modified Eagle's medium (Nissui) with 10% FCS in a humidified atmosphere containing 5% CO_2 at 37°C. The recombinant adenoviruses were purified and concentrated by sequential centrifugation in CsCl step gradients, according to the method described by Miyake and colleagues [15]. The infectious titres were determined by a modified endpoint cytopathic effect assay.

Cells were adjusted with medium to a concentration of 2×10^7 cells/0.2 ml. The cell suspension was injected into the thighs of severe combined immunodeficiency (SCID) mice (average body weight: 30 g). In a preliminary experiment, 6 weeks after the implantation, the tumour size, which was determined by the product of the maximum diameter and the length perpendicular to the maximum diameter, reached approximately 1 cm^2. The following experiments were performed using SCID mice 6 weeks after implantation of the cells.

To determine the efficiency of the recombinant adenovirus to transduce cells, 0.5×10^9, 1×10^9 and 2×10^9 plaque-forming units (PFU)/0.2 ml AxCA*LacZ* were injected intratumorally and the transduced cells were detected by β-galactosidase staining. The SCID mice were sacrificed 48 h after injection, and the tumours were removed. Each tumour specimen was divided into five pieces for each tumour because they were large. The specimens were rinsed with phosphate-buffered saline (PBS) and fixed with 2% formaldehyde and 0.2% glutaraldehyde in PBS at 4°C, then frozen with liquid nitrogen and cut into 4 μm sections. Each sample was incubated for 1 h at 37°C with X-Gal solution consisting of 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM $MgCl_2$ and 1 mg/ml X-Gal in PBS. The magnitude of staining was quantitated by counting the percentage of blue cells, and the results were consistent with all pieces in depth. The efficiency of the recombinant adenovirus to transduce both SK-OV-3 cells and KF cells was 100% for over 1×10^9 PFU/0.2 ml. The following experimental dose of AxCA*TP53* was then determined at 1×10^9 PFU/0.2 ml.

Four groups containing five SCID mice each were assigned evenly according to treatment: no treatment, CDDP alone, AxCA*TP53* alone and combination treatment of AxCA*TP53* and CDDP. AxCA*TP53* was injected intratumorally and CDDP of 1.5 mg/kg was injected intraperitoneally. The dose of CDDP, which was approximately 20% of the maximum tolerated dose [16], matched the standard clinical dose (50–75 mg/m^2). The mice receiving the combination treatment were injected simultaneously with AxCA*TP53* and CDDP. To examine the effect of the administration sequence of AxCA*TP53* and CDDP, we also added two groups each with five mice that received the following treatments: AxCA *TP53* given 24 h after CDDP and CDDP given 24 h after AxCA*TP53* in mice with SK-OV-3 tumours.

Additionally, to evaluate a non-specific adenoviral effect, 1×10^9 PFU/0.2 ml AxCA*LacZ* was injected into both tumours with and without 1.5 mg/kg CDDP.

We used the following formula to determine relative tumour size and to eliminate the variance in tumour size: relative tumour size = tumour size on day of examination/tumour size on day when treatment was started. To evaluate the effect of tumour suppression, we also calculated the T/C ratio as a percentage, where T was the actual tumour size of the treated groups and C was the actual tumour size of the controls.

Because previous studies indicated that p53 protein is expressed at 24 h and disappears 14 days after infection of SK-OV-3 cells with AxCA*TP53* [10], we sacrificed the SCID mice 14 days after treatment and performed the following histological analyses.

Apoptotic cells were detected by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) method using the *in situ* apoptosis detection kit (Oncor, Gaithersburg, MD, USA). Specimens were deparaffinised in xylene and hydrated in various percentages of alcohol. Each tissue section was treated with 40 μg/ml proteinase K for 30 min at 37°C and soaked for 30 min in 2% H_2O_2 to block endogenous peroxidase at room temperature. The slides were incubated with a labelling-reaction

mixture containing fluorescein-labelled nucleotides and terminal deoxynucleotidyl transferase (TdT) for 2 h at 37°C, and treated with 1:2 diluted antifluorescein antibody conjugated with peroxidase for 30 min at 37°C. Then peroxidase was visualised by the diaminobenzidine method. Counterstaining was performed with methyl green. The total cell count was 1000 cells in each specimen. The labelled and unlabelled cells were counted in five hyper views (×400 views). The apoptotic index (AI) was defined as follows: AI (%) = 100×apoptotic cells/1000 cells.

To detect proliferating cell nuclear antigen (PCNA) and Bax protein, immunohistochemical staining by the streptavidin–biotin–peroxidase complex method was done using paraffin-embedded tissues. Samples were heated in a microwave oven for 20 min at 94°C. The anti-PCNA monoclonal antibody and the anti-Bax polyclonal antibody were PC10 (Novocastra Lab, Newcastle, UK) and N-20 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), respectively. The antibodies were used at a 1/50 dilution (PCNA) and 1/100 dilution (Bax) in deionised water. The labelled and unlabelled cells on PCNA staining slides were counted in five hyper views. The total cell count was 500 cells in each specimen. The PCNA labelling index (LI) was determined by the following formula: LI (%) = 100×labelled cell/labelled and unlabelled cells. Omission of the primary antibody resulted in negative staining.

All experiments were performed in duplicate. Values are expressed as mean±standard deviation (S.D.). All data were compared by two-way analysis of variance with the Mann–Whitney test for quantitative data. A value of $P < 0.05$ was considered statistically significant.

3. Results

Post-treatment relative tumour size of SK-OV-3 tumours implanted in SCID mice are shown in Fig. 1. The maximum suppression of relative tumour size was observed 9 days after treatment in each group (1.06±0.08 for AxCA*TP53* alone, 0.95±0.07 for CDDP alone and 0.77±0.05 for combination treatment). The T/C ratios (%) of the SK-OV-3 tumours on day 9 were 62.4% for the tumours treated with AxCA*TP53* alone, 54.1% for the tumours treated with CDDP alone and 42.4% for the tumours treated with combination treatment. Thereafter, tumours grew gradually in each group, and the mean values of the tumour size on day 14 were 2.30±0.06 cm^2 for no treatment, 1.93±0.08 cm^2 for AxCA*TP53* alone, 1.86±0.31 cm^2 for CDDP alone and 1.22±0.04 cm^2 for the combination treatment. Each treatment significantly suppressed tumour growth. Notably, the inhibitory effect of the combination treatment on tumour growth was significant, compared with a single treatment of either CDDP or AxCA*TP53* (Fig. 1). Differences in the sequence of administration of AxCA*TP53* and CDDP did not affect the growth rate of the tumour on day 14 (1.10±0.06 for AxCA*TP53* 24 h after CDDP and 0.83±0.08 for CDDP 24 h after AxCA*TP53*) (Fig. 2).

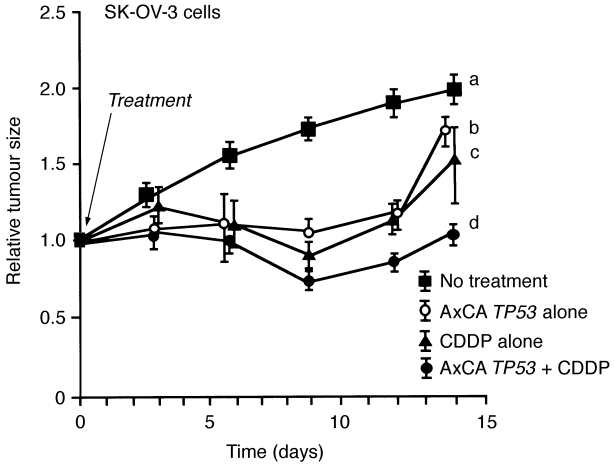

Fig. 1. Relative tumour size of SK-OV-3 tumour after treatment. a versus b, c, d: $P < 0.01$, d versus b, c: $P < 0.01$.

KF tumours grew gradually in each group and the growth rates of KF tumours on day 14 were 2.32±0.08 for no treatment, 2.05±0.07 for AxCA*TP53* alone, 1.72±0.06 for CDDP alone and 1.21±0.06 for combination treatment (Fig. 3). Mean values of tumour sizes on day 14 were 2.71±0.09, 2.34±0.09, 2.03±0.07 and 1.36±0.07 cm^2, respectively. The inhibitory effect of combination treatment on the growth of KF tumours was significant, but merely additive rather than synergistic. We also observed that, although AxCA*TP53* alone also suppressed tumour growth, that suppression was not significantly greater than in untreated tumours.

The relative sizes of SK-OV-3 tumours after treatment with AxCA*LacZ* are shown in Fig. 4. AxCA*LacZ* did not significantly affect the relative tumour size in both the SK-OV-3 tumours with and without CDDP. The same was observed in the KF tumours. No mice in

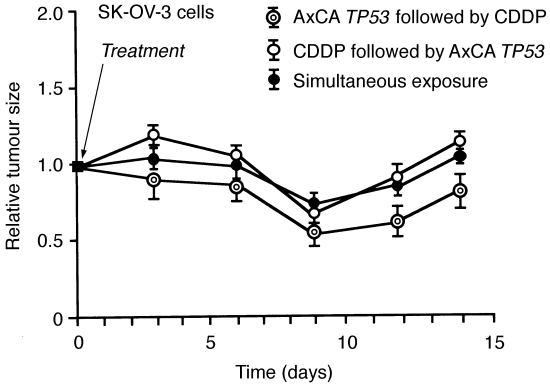

Fig. 2. Relative tumour size of SK-OV-3 tumour after different sequences of treatment administration.

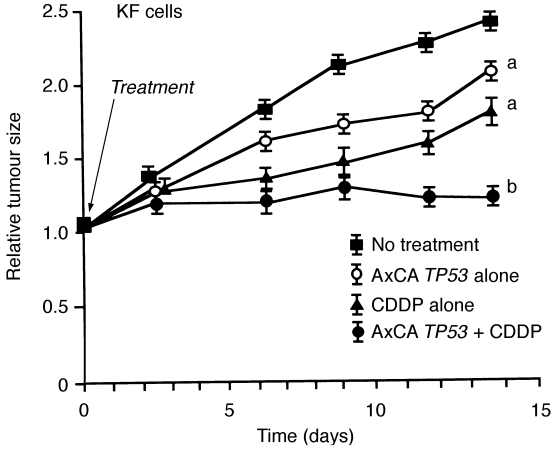

Fig. 3. Relative tumour size of KF tumour after treatment. a versus b: $P < 0.01$.

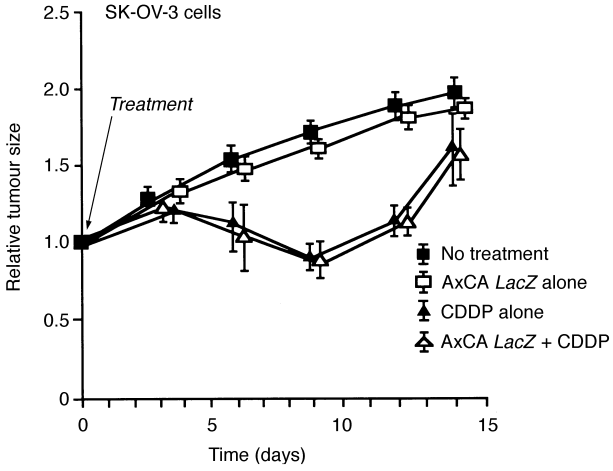

Fig. 4. Relative tumour size of SK-OV-3 tumours after treatment with AxCA*LacZ*.

this study died of toxicity from the treatment. Body weight loss (%), determined by the following formula: 100×(body weight before treatment−body weight 14 days after treatment)/body weight before treatment, was 0% in mice treated with AxCA*TP53* alone, and 6–10% in those treated with CDDP alone or with a combination of AxCA*TP53* and CDDP.

Fig. 5 shows a representative case of staining for TUNEL and PCNA in a SK-OV-3 tumours 14 days after combination treatment. AI significantly increased after each treatment reaching a maximum 9 days after treatment (data not shown), and this increase was most prominent in the combination treatment in the SK-OV-3 tumours. There were no significant differences in AI between days 9 and 14 (data not shown). In contrast, AI after transfection of AxCA*TP53* did not differ from that before treatment in the KF tumours, although the combination treatment showed the highest AI (Table 1).

LI slightly decreased after each treatment in both cells (Table 2).

The expression of Bax protein in the tumours, as estimated by immunohistochemical staining, was weak in the SK-OV-3 tumours with no treatment, and we observed that CDDP alone did not change the expression (data not shown). However, the expression of Bax protein strengthened after *TP53* gene transfection (Fig. 6). In KF tumours, the expression of Bax protein was the same before and after *TP53* gene transfection.

4. Discussion

Recent studies on the combination effect of *TP53* gene transfection and DNA-damaging agents have been reported [17–20]. *TP53* gene transfection increased the cytotoxity of CDDP in glioblastoma cells with mutated *TP53* and in lung cancer cells with mutations or deletions of the gene [19,20]. We also found that combination treatment with AxCA*TP53* and CDDP significantly suppressed tumour growth of SK-OV-3, compared with a single treatment of either AxCA*TP53* or CDDP. The maximum suppression of relative tumour size was observed 9 days after treatment, followed by a gradual tumour growth in each group. In particular, rapid growth of SK-OV-3 tumours was observed between days 12 and 14 after a single treatment of either AxCA*TP53* or CDDP. As a result, tumour sizes did not differ between any treatments and no treatment 21–25 days after treatment. This phenomenon may be explained by the degree of p53 expression and CDDP accumulation in the tumour cells.

Examining tumour suppression after administering 3.0 mg/kg CDDP, we found that suppression did not differ significantly from that of tumours treated with 1.5 mg/kg CDDP (data not shown). Additionally, AxCA-*LacZ* did not affect growth suppression in both tumours with and without CDDP. These results support our

Table 1
Apoptotic index (%) after each treatment

Treatment	SK-OV-3 cells	KF cells
No treatment	0.6±0.4[a]	0.9±0.4[a]
AxCA*TP53* alone	1.6±0.3[b]	1.2±0.3[a]
CDDP alone	2.4±0.2[b]	7.1±3.2[b]
Combination treatment	3.9±0.8[c]	8.4±0.5[b]

CDDP, cisplatin. a versus b, c: $P < 0.05$; b versus c: $P < 0.05$.

Table 2
Proliferating cell nuclear antigen (PCNA) labelling index (%) after each treatment

Treatment	SK-OV-3 cells	KF cells
No treatment	50.6±22.0	46.3±19.6
CDDP alone	47.8±21.3	43.3±18.6
AxCA*TP53* alone	48.7±20.6	39.8±18.6
Combination treatment	46.7±22.8	35.8±20.3

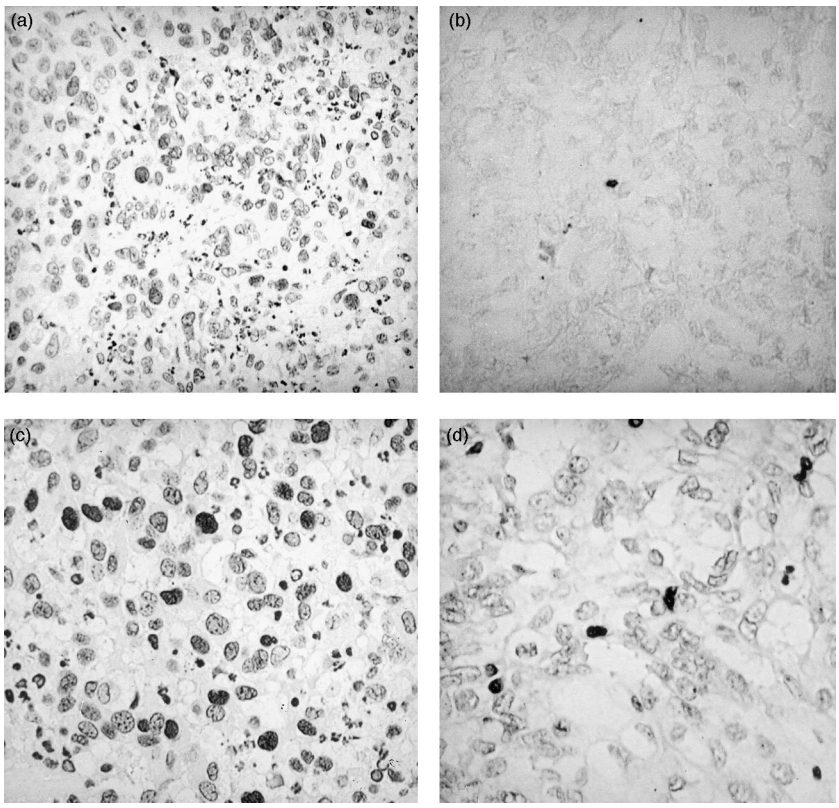

Fig. 5. A representative case of immunohistochemical staining for terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) and PCNA in tumours 14 days after treatment. (a) proliferating cell nuclear antigen (PCNA) in tumour without treatment; (b) TUNEL in tumour without treatment; (c) PCNA in tumour with combination treatment of AxCA*TP53* and CDDP; and (d) TUNEL in tumour with combination treatment of AxCA*TP53* and cisplatin (CDDP).

previous *in vitro* findings showing synergistic interaction of *TP53* and CDDP [10].

It is not clear why *TP53* transfection enhances the efficacy of DNA damaging agents such as CDDP. p53 protein is a transcriptional regulator of the cellular response to DNA damage [21]. Several studies have shown the induction of p53 by DNA-damaging agents [13,22–24]. An accumulation of p53 by cytotoxic agents including radiation delays cell growth to allow repair processes [25]. It is important in understanding *TP53* gene therapy to clarify whether and how the transfection of *TP53* enhances apoptosis or DNA repair. To

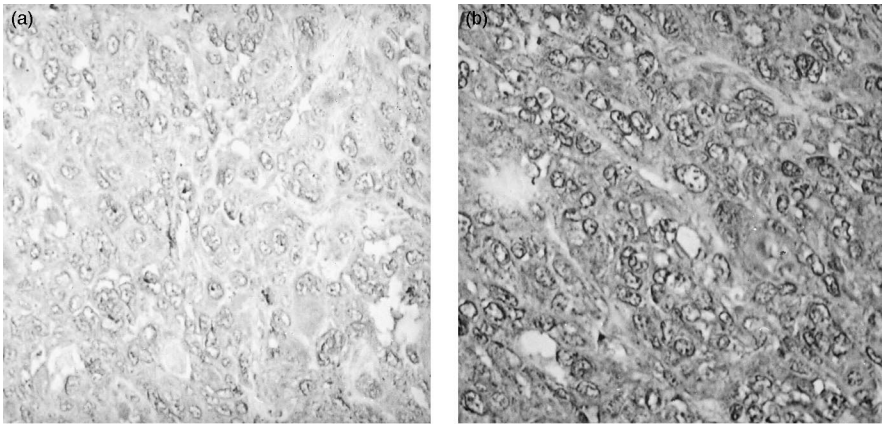

Fig. 6. Immunohistochemical staining of Bax protein in SK-OV-3 tumours 14 days after treatment. No treatment (a) and combination treatment of AxCA*TP53* and cisplatin (CDDP) (b).

our knowledge, this is the first report to demonstrate p53-dependent apoptosis in an ovarian cancer xenograft following transfection of *TP53*.

To examine p53-dependent apoptosis, we used the TUNEL assay and immunohistochemical staining for the PCNA and Bax proteins. Although the TUNEL assay is commonly used to detect apoptotic cells, one must be aware of possible false positive results. In this study, we also examined PCNA staining, a marker of cell proliferation, which is negative in TUNEL-positive cells. AI gradually increased, reached a maximum 9 days after each treatment, and then decreased slightly. AI was significantly high and LI was relatively low in an ovarian cancer xenograft with a deletion of the *TP53* gene receiving combination treatment, compared with a single treatment of either CDDP or AxCA*TP53*. PCNA, an auxiliary protein of DNA polymerase, is expressed in association with DNA repair rather than with proliferation [26,27]. These findings indicated that transfection of a wild-type *TP53* gene induces apoptosis, but does not enhance the DNA repair system in ovarian cancer cells harbouring a deletion of *TP53*. Interestingly, AI continued to be significantly higher in tumours following combination treatment compared with single treatments 14 days after treatment when p53 was not detectable. This finding may support the previous evidence that a transiently high level of wt-p53 expression was sufficient to initiate the cytotoxic programme in cancer cells [11].

p53 is a direct transcriptional activator of the *Bax* gene. Bax plays a role in the regulation of apoptosis [28,29]. Administration of CDDP alone increased AI, but did not change the expression of Bax in the SK-OV-3 tumours. This finding indicates that the SK-OV-3 cell has a p53-independent pathway for apoptosis. The expression of Bax increased following transfection of AxCA*TP53*. Thus, the appearance of apoptosis through a p53-dependent pathway may be a mechanism of the synergistic interaction of *TP53* and CDDP in SK-OV-3 tumours, whereas the expression of Bax protein did not differ before and after *TP53* gene transfection in KF tumours, indicating that the mechanism of tumour suppression in tumour cells with and without wild-type *TP53* may differ.

Furthermore, the present study showed that the inhibitory effect of combination treatment on tumour growth was significant in KF tumours which have a wild-type *TP53*. In KF tumours, AI after transfection of AxCA*TP53* did not differ from that before treatment, and the expression of Bax protein was the same before and after *TP53* gene transfection. This further supports the theory that the mechanism of tumour suppression in tumour cells with and without the wild-type *TP53* gene differs. In the literature, the increase in p53 levels in cells enhances their sensitivity to DNA-damaging agents, although the mechanism is not clear [13,30]. This finding suggests that the combination of *TP53* gene transfection and CDDP may be more effective than CDDP alone in tumours with the wild-type *TP53* and may be a potential strategy for the treatment of CDDP-resistant ovarian cancer.

The effective sequence of CDDP administration and *TP53* gene transfer has been controversial. A lung cancer study showed that the most effective regimen was CDDP given 2 days before the transfection of *TP53*, because CDDP enhanced the expression of *TP53* [20]. In contrast, another study involving lung cancer cells indicated that *TP53* gene transfection before CDDP was more effective than simultaneous exposure to adenovirus-vector with *TP53* and CDDP [11]. We also investigated the effect of sequence of administration of the two agents in a tumour xenograft. The trend of our data suggested a possible slight benefit when the virus was given before CDDP, although no statistical significance was claimed. If the appearance of apoptosis through a p53-dependent pathway is a mechanism of the synergistic interaction of *TP53* and CDDP, CDDP administration after *TP53* gene transfection may be rational in tumours with a deletion of *TP53*. Determining an effective sequence of anticancer drug and *TP53* gene transfer in a tumour xenograft is difficult, because of several factors, such as the delivery system and variations in the accumulation of CDDP and p53 protein among tumour cells, affects the combination effect. However, the sequence of administration of CDDP and *TP53* gene transfer is attractive and clinically applicable.

Whilst further study is needed to determine a more effective strategy for *TP53* gene therapy for ovarian cancer using sequential CDDP administration and *TP53* gene transfer, the present study suggests that combination therapy of AxCA*TP53* and CDDP may be a new strategy for treating chemoresistant ovarian cancer with and without wild-type *TP53*.

References

1. Boente M, Hurteau J, Rodriguez GC, Bast Jr RC, Berchuck A. The biology of ovarian cancer. *Curr Opin Oncol* 1993, **5**, 900–907.
2. Perez RP, Hamilton TC, Ozols RF, Young RC. Mechanisms and modulation of resistance to chemotherapy in ovarian cancer. *Cancer* 1993, **71**, 1571–1580.
3. Kigawa J, Minagawa Y, Ishihara H, Kanamori Y, Terakawa N. Tumor DNA ploidy and prognosis of patients with serous cystadenocarcinoma of the ovary. *Cancer* 1993, **2**, 804–808.
4. Muggia FM. Cisplatin update. *Semin Oncol* 1991, **18**, 1–4.
5. Thigpen JT, Bertelsen K, Eisenhauer EA, Hacker NF, Lund B, Sessa C. Long-term follow-up of patients with advanced ovarian carcinoma treated with chemotherapy. *Ann Oncol* 1993, **4**, 35–40.
6. Anderson SC, Johnson DE, Harris MP, *et al*. p53 gene therapy in a rat model of hepatocellular carcinoma: intra-arterial delivery of a recombinant adenovirus. *Clin Cancer Res* 1998, **4**, 1649–1659.
7. Nielsen LL, Dell J, Maxwell E, Armstrong L, Maneval D, Catino JJ. Efficacy of p53 adenovirus-mediated gene therapy against human breast cancer xenografts. *Cancer Gene Ther* 1997, **4**, 129–138.

8. Qazilbash MH, Xiao X, Seth P, Cowan KH, Walsh CE. Cancer gene therapy using a novel adeno-associated virus vector expressing human wild-type p53. *Gene Ther* 1997, **4**, 675–682.
9. Kock H, Harris MP, Anderson SC, *et al*. Adenovirus-mediated p53 gene transfer suppresses growth of human glioblastoma cells *in vitro* and *in vivo*. *Int J Cancer* 1996, **67**, 808–815.
10. Kanamori Y, Kigawa J, Minagawa Y, *et al*. A newly developed adenovirus-mediated transfer of a wild-type p53 gene increases sensitivity to cis-diamminedichloroplatinum (II) in p53-deleted ovarian cancer cells. *Eur J Cancer* 1998, **34**, 1802–1806.
11. Fujiwara T, Grimm EA, Mukhopadhyay T, Zhang WW, Owen-Schaub LB, Roth JA. Induction of chemosensitivity in human lung cancer cells *in vivo* by adenovirus-mediated transfer of the wild-type p53 gene. *Cancer Res* 1994, **54**, 2287–2291.
12. Kastan MB, Canman CE, Leonard CJ. p53 cell cycle control and apoptosis: implications for cancer. *Cancer Metast Rev* 1995, **14**, 3–15.
13. Fritsche M, Haessler C, Brandner G. Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene* 1993, **8**, 307–318.
14. Okada M, Kigawa J, Minagawa Y, *et al*. Alterations of the p53 gene and p53 protein expression in epithelial ovarian cancer before and after chemotherapy. *Int J Clin Oncol* 1998, **3**, 240–246.
15. Miyake S, Makimura M, Kanegae Y, *et al*. Efficient generation of recombinant adenoviruses using adenovirus DNA-terminal protein complex and a cosmid bearing the full-length virus genome. *Proc Natl Acad Sci USA* 1996, **93**, 1320–1324.
16. Kubota T, Nakada M, Tsuyuki K, *et al*. Cell kinetics and chemosensitivity of human carcinomas serially transplanted into nude mice. *Jpn J Cancer Res* 1986, **77**, 502–507.
17. Gjerset RA, Turla ST, Sobol RE, *et al*. Use of wild-type p53 to achieve complete treatment sensitization of tumor cells expressing endogenous mutant p53. *Mol Carcinogen* 995, **14**, 275–285.
18. Lu SJ, Man S, Bani MR, *et al*. Retroviral insertional mutagenesis as a strategy for the identification of genes associated with cis-diamminedichloroplatinum (II) resistance. *Cancer Res* 1995, **55**, 1139–1145.
19. Nielsen LL, Lipari P, Dell J, Gurnani M, Hajian G. Adenovirus-mediated p53 gene therapy and paclitaxel have synergistic efficacy in models of human head and neck, ovarian, prostate, and breast cancer. *Clin Cancer Res* 1998, **4**, 835–846.
20. Nguyen DM, Spitz FR, Yen N, Cristiano RJ, Roth JA. Gene therapy for lung cancer: enhancement of tumor suppression by a combination of sequential systemic cisplatin and adenovirus-mediated p53 gene transfer. *Thorac Cardiovasc Surg* 1996, **112**, 1372–1377.
21. Lowe SW, Bodis S, McClatchey A, *et al*. p53 status and the efficacy of cancer therapy *in vivo*. *Science* 1994, **266**, 807–810.
22. Landesman Y, Bringold F, Kimchi A. p53 undergoes eitopic changes in vitro by sodium-vanadate. *Oncogene* 1994, **9**, 1241–1245.
23. Righetti SC, Della TG, Pilotti S, *et al*. A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. *Cancer Res* 1996, **56**, 689–693.
24. Williams GT, Smith CA. Molecular regulation of apoptosis: genetic controls on cell death. *Cell* 1993, **74**, 777–779.
25. Maestro R, Dolcetti R, Gasparotto D, *et al*. High frequency of p53 gene alterations associated with protein overexpression in human squamous cell carcinoma of the larynx. *Oncogene* 1992, **7**, 1159–1166.
26. Deb S, Jackson CT, Subler MA, Martin DW. Modulation of cellular and viral promoters by mutant human p53 proteins found in tumor cells. *J Virol* 1992, **66**, 6164–6170.
27. Gorgoulis V, Zoumpourlis V, Rassidakis G, *et al*. Molecular analysis of p53 gene in laryngeal premalignant and malignant lesions. p53 protein immunohistochemical expression is positively related to proliferating cell nuclear antigen labeling index. *Virchows Arch* 1995, **426**, 339–344.
28. Jonathan J, Herod JJ, Eliopoulos AG, *et al*. The prognostic significance of Bcl-2 and p53 expression in ovarian cancer. *Cancer Res* 1996, **56**, 2178–2184.
29. Perego P, Giarola M, Righetti S, *et al*. Association between cisplatin resistance and mutation of p53 gene and reduced bax expression in ovarian carcinoma cell systems. *Cancer Res* 1996, **56**, 556–562.
30. Zhan Q, Carrier F, Fornace Jr AJ. Induction of cellular p53 activity by DNA-damaging agents and growth arrest. *Mol Cell Biol* 1993, **13**, 4242–4250.