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Sensitivity to paclitaxel is not related to p53-dependent apoptosis in ovarian cancer cells

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Abstract

We conducted this study to determine whether the sensitivity of ovarian cancer cells to paclitaxel (PTX) relates to cells undergoing p53-dependent apoptosis. Human ovarian adenocarcinoma cell lines (SK-OV-3, KF and KP cells) were used in this study. In SK-OV-3 and KP cells, which have a homozygous deletion of the *TP53* gene, wild-type *TP53* gene-transduction markedly enhanced the sensitivity to cisplatin (CDDP), but did not enhance the sensitivity to PTX. In all cells, the apoptotic index was increased by CDDP or PTX. After exposure to CDDP, p53 and Bax protein expression increased and Bcl-xL expression decreased in the KF cells and *TP53* gene-transducted SK-OV-3 cells. However, these proteins did not change in KP cells. Therefore, the role of p53 in CDDP-induced apoptosis depends upon the cell type. In contrast, *TP53* gene status did not correlate with PTX-induced cytotoxicity in any of the cell lines with differing apoptotic pathways. In conclusion, the sensitivity to PTX may not be related to p53-dependent apoptosis in ovarian cancer cells.

Keywords: Paclitaxel; Cisplatin; *TP53* gene; Apoptosis; Ovarian cancer

1. Introduction

Platinum-based combination chemotherapy, currently the standard treatment for ovarian cancer, has achieved a high response rate [1], but it is limited by the development of resistance to chemotherapy [2]. Many mechanisms have been postulated to explain this resistance, including decreased drug accumulation, increased cellular detoxification by proteins, such as glutathione (GSH) and increased DNA damage due to an impairment in repair mechanisms [3–8]. Recent studies suggest that the chemosensitivity of the cells may be related to the ability of cells to undergo apoptosis [9,10]. Several studies have shown that the *TP53* gene has a critical role in executing cell death in response to cytotoxic drugs and that mutations in *TP53* are associated with the lack of response to these agents [10–14]. *TP53* encodes a cell cycle checkpoint protein that functions in the G1 phase of the cell cycle and has a pivotal role in inducing apoptosis [11]. In a previous study, we developed a recombinant adenovirus carrying a wild-type *TP53* gene (AxCA*TP53*) and showed that p53 transduction increased the sensitivity of *TP53*-deficient ovarian cancer cells to cisplatin (CDDP) via the induction of apoptosis [14]. Bax protein, which is directly regulated by *TP53*, heterodimerises with a family of Bcl-2 proteins and promotes apoptosis. Bcl-xL, a functional and structural homologue of Bcl-2, provides protection from apoptosis [15–17]. The expression of Bcl-2 is known to be higher in normal tissues, whereas the expression of Bcl-xL is higher in ovarian cancer cells [18,19].

Paclitaxel (PTX), an antitumour drug that promotes tubulin polymerisation, changes the dynamic equilibrium in the assembling and disassembling of microtubules, disrupts the formation of the normal spindle during metaphase and causes the blockade of dividing cells [20]. Since PTX exhibited no cross-resistance or collateral sensitivity in CDDP-resistant cell lines, this drug is considered a useful agent for treating ovarian cancer [21]. Although several studies examined the role of *TP53* in determining cellular responses to PTX, the results have been contradictory [22–26].

We conducted this study to determine whether and how p53-dependent apoptosis affects the sensitivity of ovarian cancer cells to PTX.

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2. Materials and methods

2.1. Cell line and adenoviral-mediated gene transfer

A human ovarian adenocarcinoma cell line, SK-OV-3, that has a homozygous deletion of *TP53*, was obtained from the American Type Culture Collection. SK-OV-3 cells were maintained in minimum essential medium (Nissui, Tokyo, Japan), with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO_2 at 37°C. A parental human ovarian adenocarcinoma cell line (KF), which has wild-type *TP53*, was kindly provided by Dr Kikuchi, National Defense Medical College. In KP, a homozygous deletion of *TP53* from the KF cells has spontaneously occurred. These cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui, Tokyo, Japan) with 10% FBS in a humidified atmosphere containing 5% CO_2 at 37°C.

Wild-type *TP53* was transfected into both SK-OV-3 and KP cells by means of a recombinant adenovirus that contained the wild-type *TP53* gene (AxCA*TP53*). A recombinant adenovirus AxCA*LacZ*, encoding for the bacterial *LacZ* gene under the control of the CAG promoter, was used to assess the efficiency of the adenoviral-mediated gene transfer. To determine the efficiency of the recombinant adenovirus to transduce SK-OV-3 or KP cells, the cells were infected with AxCA*LacZ*, and the transduced cells were detected by β-galactosidase staining. Briefly, those cells were seeded in an 8-well chamber slide (Nunc, Roskilde, Denmark). The number of seeded cells was 30 000 cells/well, and the preincubation time was 4 h. The cells were then infected with AxCA*LacZ* at 6.25, 12.5, 25, 50, 100 and 200 multiplicities of infection (MOI). After 48 h, the cells were rinsed with phosphate-buffered saline (PBS) and were fixed with 2% formaldehyde and 0.2% glutaraldehyde in PBS. The sample was incubated 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 for 1 h at 37°C. The magnitude of staining was quantitated by counting the percentage of blue cells.

As a result of assessing the efficiency of adenoviral-mediated gene transfer, a 100% transduction efficacy was over 25 MOI for the SK-OV-3 cells and over 50 MOI for the KP cells. Additionally, a recombinant adenovirus that had a reverse-inserted *TP53* gene (AxCA*TP53R*) was used as a control [14].

2.2. Cell suppression and apoptosis

CDDP and PTX were obtained from Bristol-Myers Squibb, Tokyo, Japan. The effect of CDDP or PTX was evaluated by the dimethylthiazolyl-2,5-diphenyl-tetrazolium bromide (MTT) assay in the SK-OV-3 cells, KF cells and KP cells. Cells (10 000 cells/well) were seeded in 96-well plates and pre-incubated for 4 h, then incubated for 72 h after exposure to CDDP or PTX. Additionally, SK-OV-3 and KP cells were infected with either AxCA*TP53* or AxCA*TP53R*. Infectious units of each virus per cell were 25 MOI for SK-OV-3 cells and 50 MOI for KP cells. CDDP or PTX was added 1 h after infection with the virus, then the cells were incubated for 72 h. Concentrations of CDDP ranged from 0.9 to 22.2 μM, and those of PTX ranged from 7.0 to 1500 nM. The dose–response curve was plotted on a semi-log scale as a percentage of the control cell number obtained from the untreated sample. The IC_{50} was determined from the dose–response curve as a percentage of the control cell number without the drugs.

To assess apoptosis, cells (100 000 cells/well) were seeded into 28 cm^2 dishes and pre-incubated for 4 h. SK-OV-3 cells and KP cells were infected with 25 MOI and 50 MOI AxCA*TP53* or AxCA*TP53R*, respectively. CDDP or PTX of IC_{50} for each cell line was added 24 h after infection with the virus and the cells were incubated for 72 h.

Apoptotic cells were assessed morphologically by staining with Hoechst 33258 (Calbiochem-Novabiochem, San Diego, CA, USA) using cells fixed with Clarke fixative (ethanol:acetic acid = 3:1). Apoptotic index (AI) was defined as follows: AI (%) = 100 × apoptotic cells/200 cells.

2.3. p53, Bax and Bcl-xL protein

p53, Bax and Bcl-xL protein expression were determined by Western blot analysis. Forty-eight hours after exposure to the IC_{50}, CDDP or PTX-treated cells were solubilised on ice in lysis buffer (50 mM Tris–HCl, 125 mM NaCl, 0.1% NP40, 5 mM ethylenediamine tetraacetic acid, 50 mM NaF, 0.1% phenylmethyl sulphonyl fluoride and protease inhibitors including 1% lenpeptin, 10% soybean trypsin inhibitor, 1% aprotinin and 10% tosylphenylaranine chloromethyl ketone) and centrifuged at 25 000*g* for 30 min. The total protein concentration in the supernatant was measured, and samples of 60 μg protein were separated by electrophoresis on a 4–20% gradient polyacrylamide gel. The separated proteins were transferred onto a polyvinylnylidene difluoride membrane (Millipore Co., Bedford, MA, USA). Proteins were visualised with antimouse or antirabbit IgG coupled to horseradish peroxidase, using enhanced chemiluminescence (ECL) according to the manufacturer's recommendation. The primary anti-p53 monoclonal antibody was DO-7, (DAKO, Glostrup, Denmark). The anti-Bcl-xL and Bax polyclonal antibodies were L-19 and N-20 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), respectively. All antibodies were used at 1/500 dilution in 0.05% tween PBS. After ECL detection reagent staining (Amersham International, Bucks, UK), the immunoblots were

quantitated on a Macintosh Quadra 840AV computer. We used the public domain NIH Image program (written by Wayne Rasband at the US National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd, Springfield, VA 22161, USA, part number PB93-504648). A relative ratio of Bax to Bcl-xL was calculated.

2.4. Statistical analysis

All assays were performed in triplicate. Means for all data were compared by Fisher's one-way *post-hoc* test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Combination effect of AxCATP53 and CDDP or PTX

Dose–response curves of SK-OV-3, KF and KP cells after exposure to CDDP or PTX are shown in Fig. 1. IC_{50} to CDDP was 12.9 μM for non-infected or AxCA*T53R*-infected SK-OV-3 cells and 9.3 μM for AxCA*TP53*-infected cells. In contrast, IC_{50} value to PTX did not change after transduction of *TP53* gene (62 and 52 nM, respectively). IC_{50} to CDDP was 6.4 μM for non-infected or AxCA*TP53R*-infected KP cells, 3.7 μM for AxCA*TP53*-infected KP cells and 3.1 μM for KF cells. IC_{50} to PTX were 800, 750 and 130 nM, respectively.

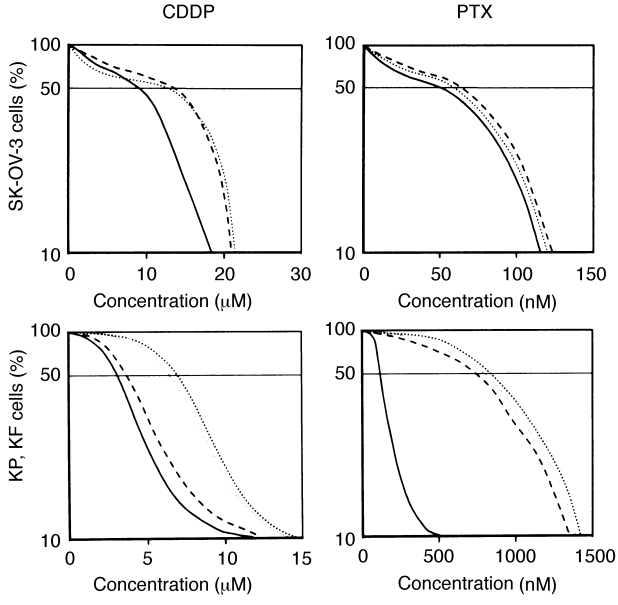

Fig. 1. Dose–response curves of CDDP or PTX in combination with AxCA*TP53*. Transduction of the *TP53* gene increased sensitivity to CDDP in SK-OV-3, KP and KF cells. Transduction of the *TP53* gene did not affect sensitivity to PTX in all cell lines. ·····, no infection; – –, 25 MOI AxCA*TP53R*; and —, 25 MOI AxCA*TP53* for SK-OV-3 cells (upper panels); ·····, no infection; - - - - -, 50 MOI AxCA*TP53R*; – –, 50 MOI AxCA*TP53* for KP cells; and —, 50 MOI AxCA*TP53* KF cells (lower panels).

AIs before and after treatment in each cell line are shown in Table 1. The AIs significantly increased after transduction of *TP53* into SK-OV-3 and KP cells. Regarding the KF series, the AI was highest for *TP53*-transfected KP, followed by KF and then KP cells. Transduction of *TP53* significantly enhanced CDDP-induced apoptosis, but did not enhance PTX-induced apoptosis in SK-OV-3 and KP cells.

3.2. p53, Bax, and Bcl-xL protein

Fig. 2 shows the expression of p53, Bax and Bcl-xL proteins in each cell line. The expression of p53 and Bax protein increased with exposure to CDDP in the KF cells, whereas, in the SK-OV-3 and KP cells, the expression of Bax protein did not change with exposure to CDDP. Bax expression increased after *TP53* gene transduction, and CDDP further enhanced the expression of Bax (and also p53) in the SK-OV-3 cells. *TP53* gene transduction into KP cells induced p53 protein expression, but CDDP did not affect Bax expression in p53-transduced KP cells. The expression of Bcl-xL protein decreased with CDDP exposure in KF and SK-OV-3 cells. A significant correlation between the AI and the ratio of Bax to Bcl-xL was observed in these cells (Fig. 3). The ratio of Bax to Bcl-xL did not correlate with AI in KP cells. PTX did not affect either the Bax expression or Bcl-xL expression in any cell lines.

4. Discussion

The response to anticancer drugs is linked to apoptosis, although the precise mechanisms of this response have not been elucidated [10–12,27,28]. In this study, we found that, regardless of the *TP53* gene status, AI increased after exposure to CDDP or PTX, and that apoptosis paralleled cytotoxity in all the examined cell lines. To determine whether and how p53-dependent apoptosis affects the sensitivity of cells to CDDP or PTX in ovarian cancer, we transfected *TP53* into p53-deficient cells, and examined the expression of apoptotic-regulators, such as the Bax and Bcl-xL proteins, which are located transcriptionally downstream in the apoptotic pathway.

TP53 gene transduction markedly enhanced the sensitivity to CDDP in SK-OV-3 and KP cells which harbour homozygous deletions of *TP53*. Because CDDP induced apoptosis in SK-OV-3 and KP cells, these cells may have a p53-independent pathway. CDDP enhanced the expression of p53 and Bax proteins in KF and *TP53* gene- transfected SK-OV-3 cells. In KF cells, CDDP-induced apoptosis mainly occurred through the p53-dependent pathway. Apoptosis occurred

Table 1
Apoptotic index (AI) in each cell line[a]

Treatment	SK-OV-3 cells		KP cells		KF cells
	No transduction	*TP53* gene transduction	No transduction	*TP53* gene transduction	
No exposure	6.7±2.9[a1]	12.2±3.9[a2]	4.3±1.1[a3]	10.6±0.5[a4]	7.3±2.4[a5]
Exposure to CDDP	33.4±2.9[b1]	48.8±1.8[b2]	26.5±4.1[b3]	39.4±3.6[b4]	27.3±4.9[b5]
Exposure to PTX	14.2±1.2[c1]	16.8±3.1[c2]	24.4±6.7[c3]	33.2±6.1[c4]	22.0±2.4[c5]

CDDP, cisplatin; PTX, paclitaxel.
[a] Data are presented as mean±standard deviation (S.D.). Five experiments were conducted. a1 versus a2, a3 versus a4; $P < 0.05$, a versus b, c; $P < 0.05$, b1 versus b2, b3 versus b4; $P < 0.05$.

through both p53-dependent and independent pathways in the *TP53* gene-transfected SK-OV-3 cells. In contrast, *TP53* gene transduction in the KP cells did not enhance Bax expression following CDDP treatment, although the gene transduction did induce p53. The Bcl-2 family, including Bcl-xL, competes for Bax preventing its homodimerisation and thereby forming heterodimers with Bcl-2 family. The ratio of Bcl-2 to Bax therefore affects cell viability [17]. Bcl-xL is one of the Bcl-2 family and has an important role in solid tumours [18]. In our study, Bcl-2 expression did not differ among all cell lines (data not shown). Interestingly, we found a significant correlation between the AI and the ratio of Bax to Bcl-xL in SK-OV-3 and KF cells, but not in KP cells. These findings suggest that the cell lines utilised different apoptotic pathways and that the role of p53 in CDDP-induced apoptosis may depend upon the cell type.

However, we found that the expression of p53, Bax or Bcl-xL proteins did not change following exposure to PTX in all of the cell lines. Additionally, transduction of *TP53* did not affect the sensitivity to PTX in SK-OV-3 cells and KP cells. The role of p53 in determining cellular responses to PTX treatment is controversial [22–26]. It has been reported that *TP53* status did not affect the sensitivity of human ovarian cancer cell lines to PTX [22,23]. In contrast, other authors have suggested that p53 function conferred sensitisation to PTX [24–26]. In the literature, depending on cell type, p53 has been shown to have no effect or to increase or decrease PTX-induced cytotoxicity [23–26,28]. The present study demonstrated that the *TP53* gene status did not correlate with PTX-induced cytotoxicity in any of cell lines. O'Connor and colleagues also reported the status of the *TP53* tumour suppressor pathway in 60 cancer cells

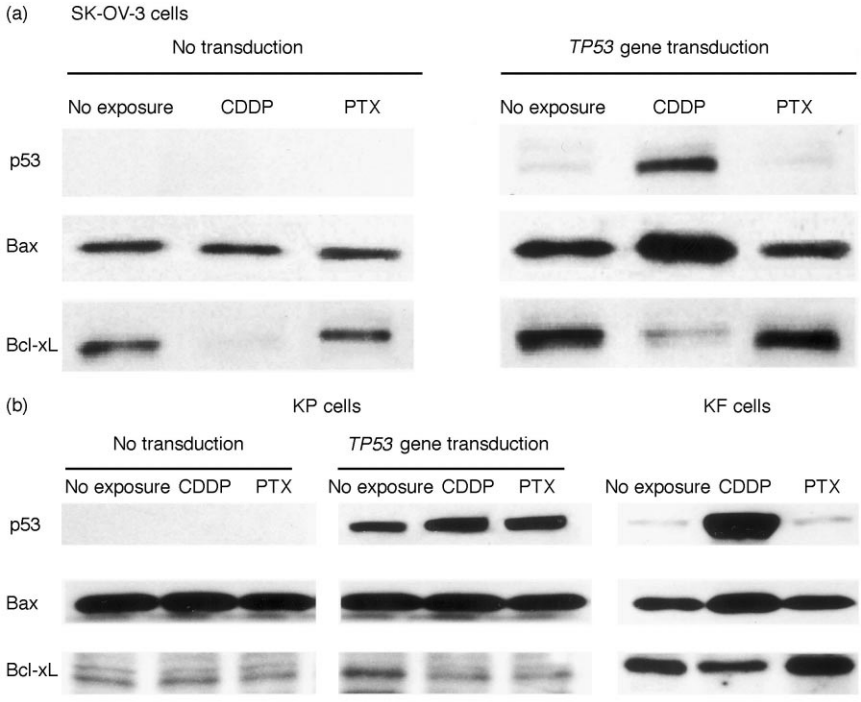

Fig. 2. (a) The expression of p53, Bax, and Bcl-xL proteins in SK-OV-3 cells. (b) The expression of p53, Bax and Bcl-xL proteins in KP and KF cells.

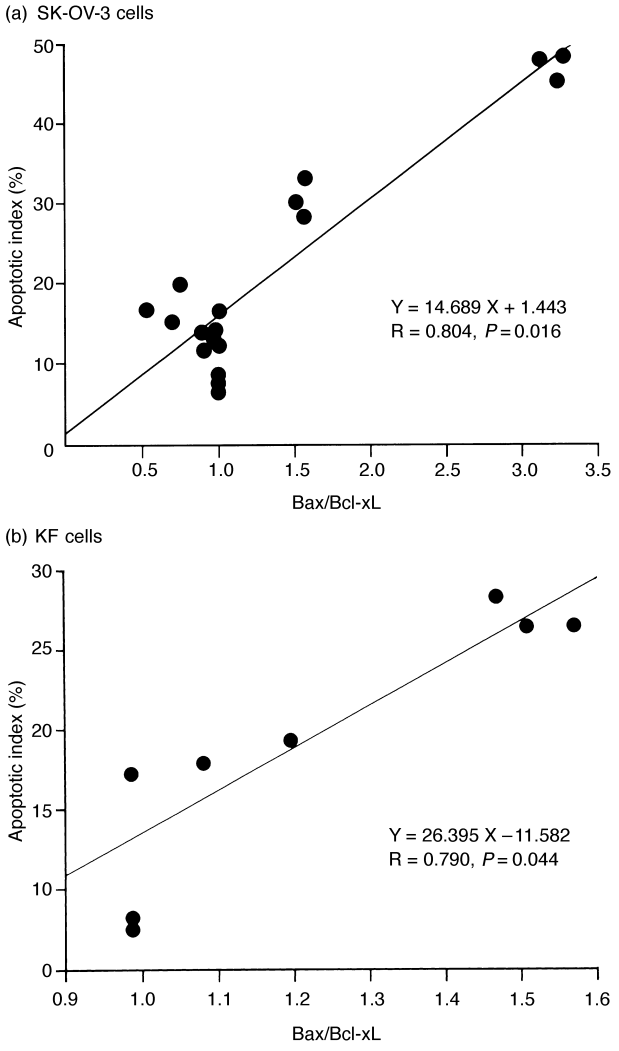

Fig. 3. Correlation between apoptotic index (AI) and the ratio of Bax to Bcl-xL in (a) SK-OV-3; and (b) KF cells. There was a significant correlation between AI and the ratio in these cells.

used in the NCI anticancer drug screen and determined the relationship between p53 status and the activity of 123 anticancer agents [29]. In their study, cell lines with defective *TP53* tended to be less sensitive to the majority of alkylating agents, CDDP, antimetabolites and topoisomerase inhibitors. In contrast, no such relationship was seen for the antimitotic agents such as PTX. Although the literature reports that the Bcl-xL protein suppressed apoptosis induced by PTX [30], the present study showed that PTX did not affect the expression in any cell lines. Additionally, it is reported that the sensitivity to PTX increased in cells where Bax is overexpressed, because the efflux of PTX was impaired in these cells [31]. In the present study, *TP53* gene transduction slightly enhanced Bax protein in SK-OV-3 cells and did not enhance it in KP cells. Moreover, the sensitivity to PTX did not change after transduction of *TP53* in both cells. These findings indicate that PTX-induced apoptosis may be p53-independent in ovarian cancer cells. Interestingly, KP cells (which have no *TP53*) appeared less sensitive to PTX after *TP53* transduction than the KF cells from which they were derived. While further study is necessary to draw conclusions, KP cells may have a mechanism of resistance to antimitotic agents such as PTX that is different from KF cells.

Nielsen and coworkers reported that adenoviral-mediated *TP53* gene therapy and PTX had synergistic efficacy in models of head, neck, ovarian, prostate and breast cancer in humans [32]. They did not examine the apoptotic pathway, but described a phenomenon in which the viral vector enhanced PTX accumulation. In contrast, we did not find that transduction of *TP53* enhanced PTX-induced apoptosis. Thus, *TP53* gene therapy does not enhance PTX-induced apoptosis, at least from the data in this study. *TP53* gene mutation frequently occurs in recurrent ovarian cancer and that alteration of the *TP53* gene status affects salvage chemotherapy [33]. We also found that p53-dependent apoptosis in tumours is strongly related to the sensitivity to CDDP-based chemotherapy in epithelial ovarian cancer [34]. The present study suggests that the sensitivity to PTX is not related to p53-dependent apoptosis. While further study is necessary, PTX-based chemotherapy may be an effective treatment for CDDP-resistant ovarian cancer containing the mutant *TP53* gene.

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