Neoplasia • Vol. 2, No. 3, May–June 2000, pp. 202–207 202

www.nature.com/neo

Driving *p53* Response to *Bax* Activation Greatly Enhances Sensitivity to Taxol by Inducing Massive Apoptosis

Paola De Feudis, Sara Vignati*, Cosmo Rossi†, Tatiana Mincioni†, Raffaella Giavazzi‡, Maurizio D'Incalci* and Massimo Broggini*†*

*Molecular Pharmacology Unit, Department of Oncology, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan, Italy; †Experimental Models and Animal Care Unit, Consorzio Mario Negri Sud, S. Maria Imbaro, Chieti, Italy; ‡Laboratory of Biology and Treatment of Metastasis, Department of Oncology, Istituto di Ricerche Farmacologiche "Mario Negri", Bergamo, Italy

Abstract

The proapoptotic gene *bax* is one of the downstream effectors of *p53*. The *p53* binding site in the *bax* promoter is less responsive to *p53* than the one in the growth arrest mediating gene *p21*. We introduced the *bax* gene under the control of 13 copies of a strong *p53* responsive element into two ovarian cancer cell lines. The clones expressing *bax* under the control of *p53* obtained from the wild-type (wt) *p53*-expressing cell line A2780 were much more sensitive (500- to 1000-fold) to the anticancer agent taxol than the parent cell line, with a higher percentage of cells undergoing apoptosis after drug treatment that was clearly *p53*-dependent and *bax*-mediated. Xenografts established in nude mice from one selected clone (A2780/C3) were more responsive to taxol than the parental line and the apoptotic response of A2780/C3 tumors was also increased after treatment. Introduction of the same plasmid into the *p53* null SKOV3 cell line did not alter the sensitivity to taxol or the induction of apoptosis. In conclusion, driving the *p53* response (after taxol treatment) by activating the *bax* gene rather than the *p21* gene results in induction of massive apoptosis, *in vitro* and *in vivo*, and greatly enhances sensitivity to the drug. *Neoplasia* (2000) **2,** 202–207.

Keywords: apoptosis, *p53*, anticancer agents, tumor xenografts, transcription.

Introduction

The product of the tumor suppressor gene *p53* is a key protein with multiple functions inside the cells. The majority of the functions of *p53* are related to its ability to bind DNA and activate the transcription of different genes [1,2]. After treatment with different damaging agents, the *p53*, which normally has a short half-life, is stabilized with an increase in its levels [3] and is activated inducing the transcription of downstream genes. Many important genes are activated by *p53*, including *WAF1*, *GADD45*, *mdm2* and *bax*. Schematically, depending on the type and extent of the damage and the cell type, the increase in *p53* causes cell cycle arrest (in either G1 or G2) or apoptosis [4–6]. Although the cell cycle arrest is thought to be mainly mediated by $p21^{WAF1}$, a potent inhibitor of cyclin-dependent kinases (cdks) [7,8], the apoptosis induced by *p53* seems to involve the activation of different genes, the proapoptotic gene *bax* being one — although not the only one — of the key players [9,10]. In some cell lines the cell cycle arrest induced by *p21* has a protective effect to the treatment of anticancer agents [6,11–14].

Introduction of the proapoptotic gene *bax* under the control of exogenous promoters into cancer cells increases apoptosis and the cellular response to anticancer drugs [15–18]. The possibility of driving the action of *p53* by activating *bax* rather than the *p21* gene should therefore increase the cancer cell's susceptibility to drug action. The *p53* responsive element in the *p21* promoter is much more potent in activating its transcription than the one in the *bax* promoter (Hardy-Bessard AC and Soussi T, personal communication) and this is probably one reason for the lower levels of *bax* than *p21* observed in some wild-type (wt) *p53*-expressing cancer cell lines after drug treatment [19,20].

We here report that introducing into a human ovarian cancer cell line expressing wt *p53* (A2780) the *bax* gene under the control of 13 copies of a strong *p53* responsive element, results in massive induction of apoptosis and increases sensitivity to taxol. The same plasmid introduced into a human ovarian cancer cell line not expressing *p53* did not change the sensitivity to taxol, indicating that the *bax*-induced apoptotic effect in the A2780 transfected cells is *p53*-dependent.

Materials and Methods

Cells and Constructs

The human ovarian cancer cell lines A2780 and SKOV3, expressing respectively wt *p53* and no *p53*, were maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS).

To construct the plasmid containing the *bax* gene under the control of 13 copies of a strong *p53* responsive element,

Address all correspondence to: Dr. Massimo Broggini, Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, 20157 Milan, Italy. E-mail: broggini@irfmn.mnegri.it

Received 30 December 1999; Accepted 14 January 2000.

the *Hin*dIII/*Eco*RI fragment of PG13luc (kindly supplied by Dr. B. Vogelstein) was inserted in the *Hin*dIII/*Eco*RI digested pBluescript II SK. This construct was digested with *Sma*I/*Xba*I and ligated with the *Sma*I/*Xba*I fragment of pUHD10.3Bax (kindly supplied by Dr. S. Bergmann) to generate pSK13BAX. The correct insertion was verified by DNA sequencing.

For stable transfection A2780 and SKOV3 cells were seeded 24 hours before transfection. Cells were cotransfected with the pSK13BAX and pSV2neo plasmids using the calcium phosphate precipitation method. pSV2neo contained a neomycin-resistant gene that allowed neomycin-resistant colonies to be selected approximately 14 days after transfection in G418-supplemented (500 μg/ml) medium.

Evaluation of Taxol-Induced Cytotoxicity

Cytotoxicity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay in 96-well plates (Nunc) at different times after treatment with different concentrations of taxol (obtained from Bristol-Meyers, dissolved in DMSO at a concentration of 1 mM and stored at -20°C). Drug concentrations inhibiting the growth by 50% (IC_{50}) were calculated after 72 hours of recovery in drug-free medium after 24 hours taxol treatment.

Evaluation of Apoptosis

To detect apoptotic cells, cells were seeded on glass coverslips in 24-well plates (25 000 cells/ml) and treated with the drug at different concentrations. After 24, 48 and 72 hours, attached cells were fixed in 70% ethanol, air dried and stained with DAPI (4′,6-diamino-2-phenylindole) and sulphorhodamine B [19]. Percentage of cells with characteristic apoptotic morphology was determined by counting different fields, each consisting of at least 100 cells.

Western Blotting Analysis

Total cell extracts were prepared from untreated or treated cells at different times after drug exposure, according to standard procedures [21]. One hundred micrograms of proteins for each sample were electrophoresed through 12% polyacrylamide-SDS gels and electroblotted onto nitrocellulose membrane (Schleicher and Schull, Germany) in transfer buffer (50 mM Tris, 100 mM glycine, 0.01% SDS, 20% methanol) for 2 hours at 50 V. Filters were stained with Ponceau red, hybridized with monoclonal antibody against *p53* (clone DO-1) or polyclonal antibodies against *bax* and *p21* (Santa Cruz Biotechnology) and detected with the ECL (enhanced chemiluminiscence) system (Amersham). The experiments were repeated at least twice in all cell lines.

Tumor Transplantation and Drug Treatment

A2780 and A2780/C3 were grown subcutaneously (s.c.) in nude mice (female NCr-nu/nu, Animal Production Colony, NCI-FCRC, Frederick, MD) for one passage to unify their growth *in vivo*. Two 3-mm tumor fragments were then implanted s.c. in the flank of nude mice and treatment (six randomized mice per group) started when the tumor

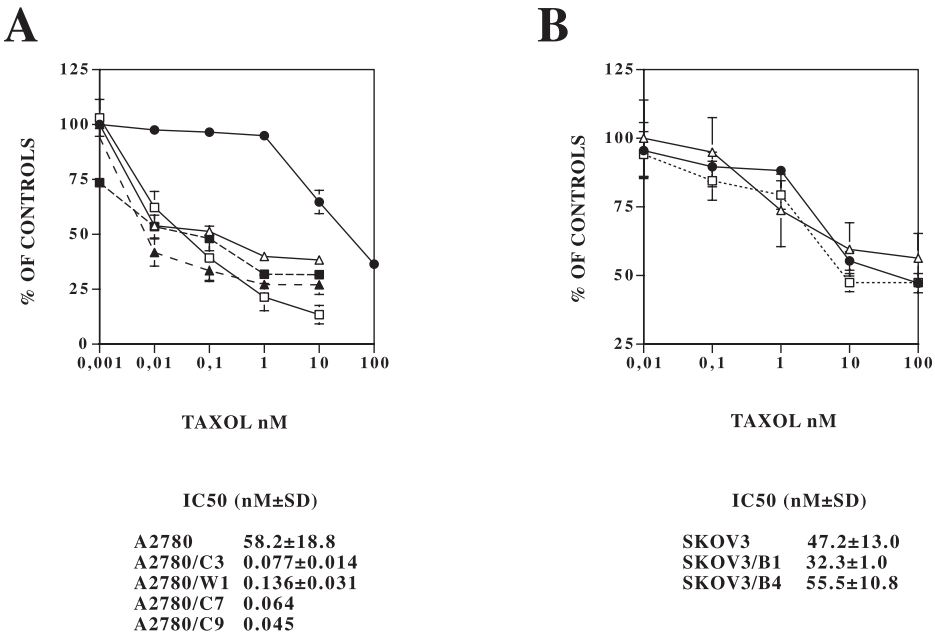

Figure 1. *Dose-dependent growth inhibition, measured by the MTT assay, induced by taxol in A2780 (●), A2780/C3 (□), A2780/W1 (△), A2780/C7 (▲) and A2780/C9 (■) cells (panel A) and SKOV3 (●), SKOV3/B1 (□) and SKOV3/B4 (△) cells (panel B) treated with different taxol concentrations for 24 hours. The IC50 values are reported for each clone as the mean ± SD of at least three independent experiments except for A2780/C7 and A2780/C9 where the IC50 is the mean of two experiments.*

reached approximately 300 mg in size. The tumor diameters were measured every 3 days with a caliper and the weights in milligrams were calculated as [length × (width)^2/2]. Taxol (dissolved in Chremophor EL) was given intravenously every 4 days for three injections at doses of 3, 6 or 12 mg/kg. Changes in tumor weight from the start of treatment (Wo) until the value at any given time (Wt) were calculated for each tumor and for each day of measurement and expressed as relative tumor weights (RTW=Wt/Wo). The optimal growth inhibition is defined as the lowest ratio of RTW of treated over control tumors × 100. With a $T/C \leq 50\%$ the treatment is considered active [22].

Three mice per group were killed 24 hours after treatment, the tumor was removed and immediately deep frozen for Southern blotting analysis and for TUNEL staining, which was performed on tissue sections using an *in situ* cell-death detection kit (Boehringer, Italy) following the manufacturer's instructions. Stained, apoptotic cells were visualized by fluorescence microscopy.

Results

In Vitro Cytotoxicity Induced by Taxol

After transfection with the pSK13BAX plasmid, different clones were obtained from A2780 and SKOV3 cells.

We first analyzed taxol-induced cytotoxicity, using the MTT assay after 24 hours treatment with different taxol concentrations (Figure 1*A*). In A2780 parental cells, cell number was significantly reduced only at taxol concentrations greater than 10 nM, whereas in the clones obtained from A2780 (A2780/C3, A2780/W1, A2780/C7 and A2780/C9) 0.01 nM of taxol already had significant activity. The taxol IC_{50} values, calculated from three independent experiments were 58.2±18.8 nM for A2780 cells and 0.077±0.014, 0.136±0.031, 0.064 and 0.045 nM respectively for the three clones A2780/C3, W1, C7 and C9. In contrast, (Figure 1, *panel B*) taxol had similar activity in the SKOV3 cell line (not expressing *p53*) and in the SKOV3 clones (B1 and B4) obtained after transfection with the same *p53*-dependent, *bax* expressing plasmid. For further characterization, two clones (A2780/C3 and SKOV3/B1) were selected.

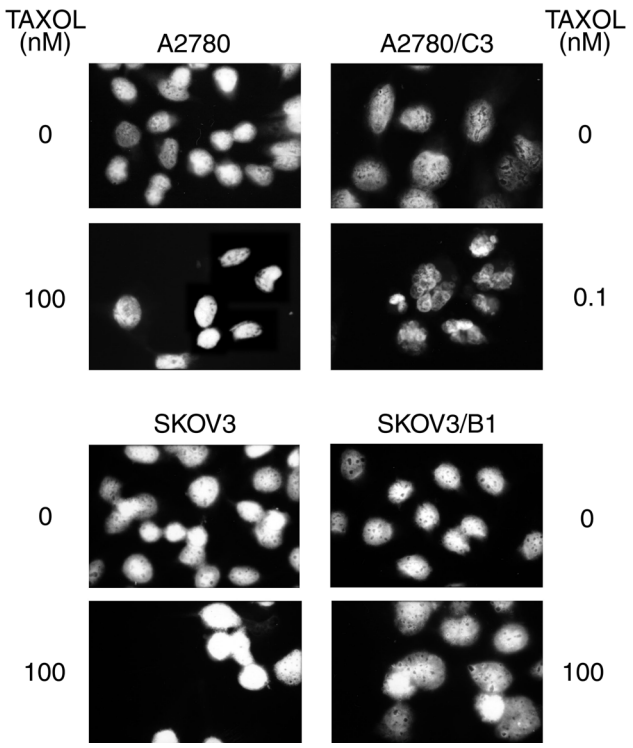

Figure 2. *DAPI-sulphorhodamine staining of A2780, A2780/C3 (upper panel), SKOV3 and SKOV3/B1 cells (lower panel) treated for 24 hours with different taxol concentrations, as indicated.*

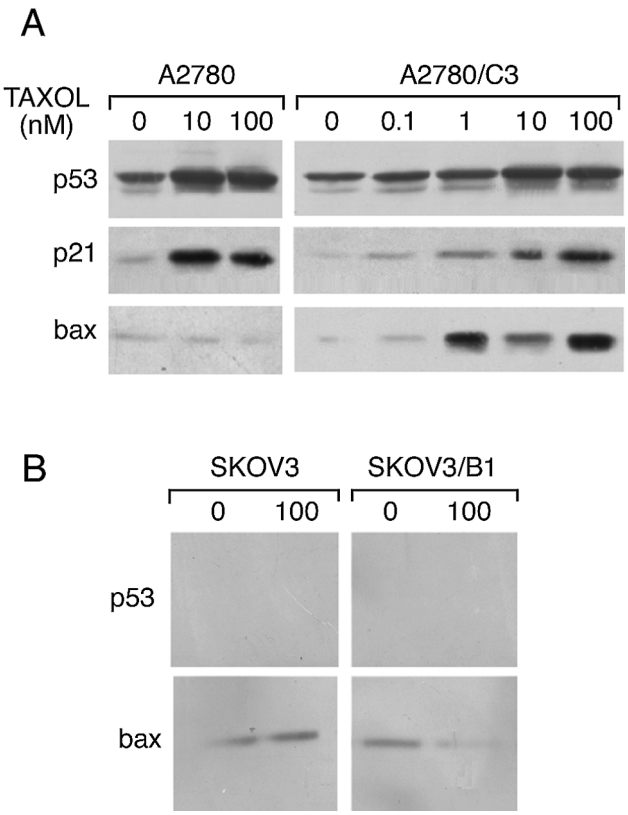

Figure 3. *Western blotting analysis of p53, bax and p21 expression in A2780 and A2780/C3 (panel A), SKOV3 and SKOV3/B1 (panel B) cells treated for 24 hours with different taxol concentrations, as indicated.*

Induction of Apoptosis and Expression of Bax

Microscopic examination of cells (after DAPI-sulphorhodamine staining) treated for 24 hours with different concentrations of taxol (Figure 2) revealed the typical morphology of apoptosis in A2780/C3 cells even at taxol concentrations as low as 0.1 nM, whereas in the parental A2780 cell line no significant apoptosis was evident even at 1000 times higher drug concentrations. The percentage of apoptotic cells in untreated A2780 cells was 2.0±1.4% and slightly increased to 13.1±8.5% 24 hours after treatment with 100 nM of taxol. By contrast, in A2780/C3 cells the

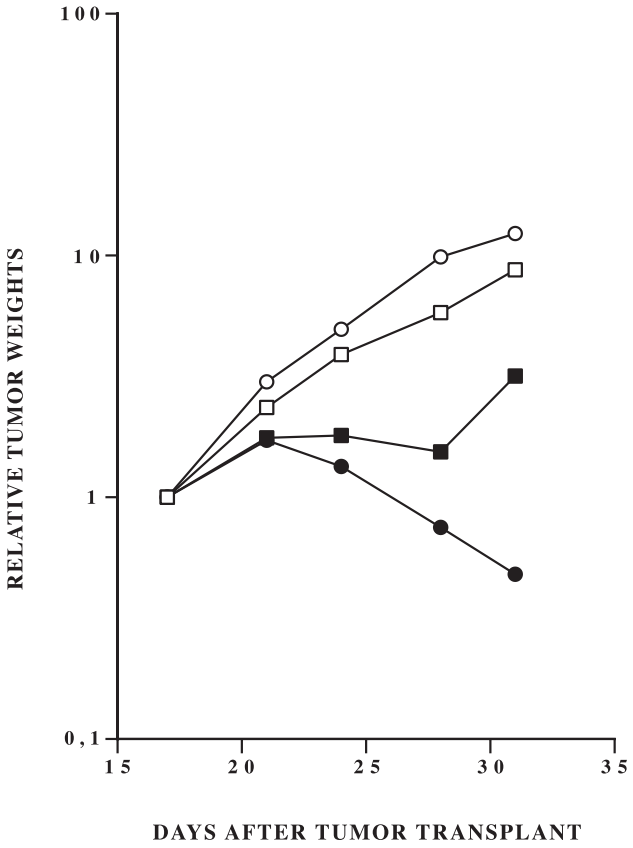

Figure 4. *Tumor growth in nude mice implanted with A2780 (■, □) or A2780/C3 (●, ○) cells and treated with vehicle (□, ○) or 12 mg/kg (■, ●) of taxol every four days for three times.*

percentage of cells undergoing apoptosis strongly increased from 2.8±2.4% (in untreated cells) to 38.7±1.7% 24 hours after treatment with a dose as low as 0.1 nM of taxol. In SKOV3 cells and in the clone SKOV3/B1 treated for 24 hours with different taxol concentrations, there were no differences between the two cell lines and no evidence of apoptosis was found even at the highest taxol concentration (100 nM). In this experimental system the percentage of cells with apoptotic morphology was always lower than 1%.

Western blot analysis of *p53* and *bax* expression in A2780, A2780/C3, SKOV3 and SKOV3/B1 cells (Figure 3, *A* and *B*) demonstrated that both A2780-derived cell lines showed an increase in the levels of *p53* after treatment with taxol. *bax*, however, was almost undetectable in the parental A2780 cells, whereas in the A2780/C3 cells the levels of this protein were strongly induced at all the taxol concentrations tested (*panel A*). The selective induction of *bax* in A2780/C3 but not in A2780 cells was already evident 6 hours after taxol treatment, whereas at 3 hours we did not detect any *p53* induction and consequently any *bax* induction in both cell lines (data not shown). In these treatment conditions, *p21* induction was similar in A2780 and A2780/C3 cells.

In SKOV3 and SKOV3/B1 cells (*panel B*) *p53* was, as expected, undetectable and the levels of *bax* in the parental SKOV3 cell line were not different from those in SKOV3/B1 cells and did not change after taxol treatment in both lines.

In Vivo Activity and Induction of Apoptosis by Taxol in p53-Dependent Bax-Expressing Clones

Both A2780 and A2780/C3 cells were transplantable in nude mice giving tumors in all the implanted animals and by Southern blot analysis we could confirm the presence of pSK13BAX in the A2780/C3 clone growing *in vivo* in nude mice.

In vehicle-treated mice, A2780 and A2780/C3 tumors grew at similar rates (reaching 1 g tumor weight in 23 and 24 days, respectively). The activity of taxol was clearly dose-dependent in the A2780/C3 tumors treated with the optimal schedule of one i.v. injection every four days for three times. The highest tested dose of 12 mg/kg gave a significant growth inhibition (*T*/*C* 4%) with 50% of tumors in partial regression (greater than 50% reduction in tumor mass) (Figure 4). At day 31, mean tumor weights in

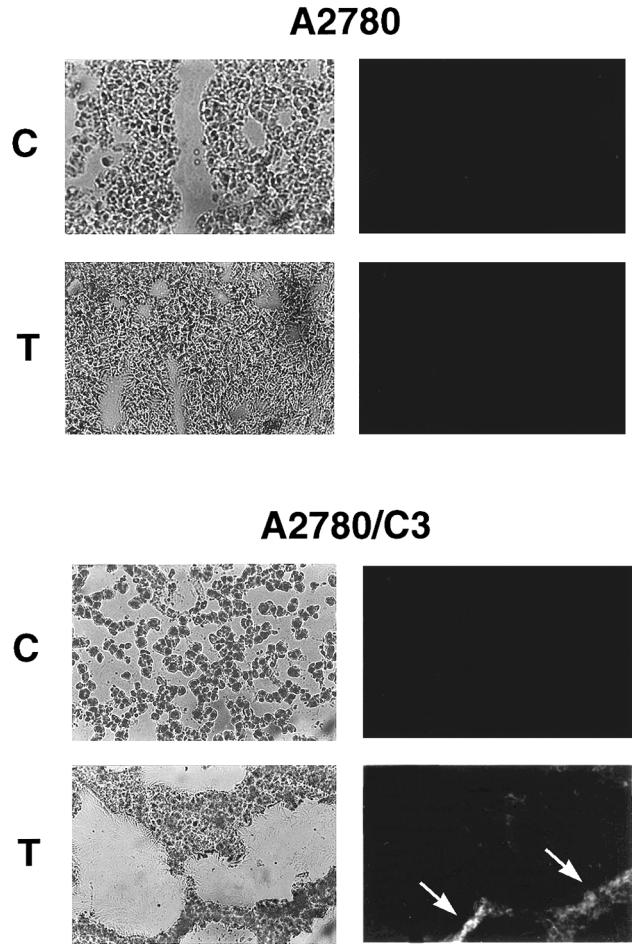

Figure 5. *TUNEL staining of A2780 and A2780/C3 tumor sections obtained 24 hours after treatment with vehicle (C) or 12 mg/kg of taxol (T). Photomicrographs of phase contrast (left panels) and the corresponding TUNEL fluorescence (right panels) are reported. Arrows indicate areas of fluorescence (white) corresponding to apoptotic cells.*

control and treated groups were 2.2±0.57 and 0.30±0.11 g respectively.

The dose of 6 mg/kg caused delay of tumor growth with a *T/C* of 24%, whereas the dose of 3 mg/kg showed a borderline efficacy (*T/C*=48%) (data not shown).

On A2780 parental tumors, taxol caused a moderate inhibition (*T/C*=27%) at the highest dose of 12 mg/kg (Figure 4) and it was not active (*T/C*-55%) at 6 mg/kg (data not shown).

Direct evidence of *in vivo* taxol-induced apoptosis was obtained by TUNEL analysis of tumor tissues obtained 24 hours after a single treatment with 12 mg/kg of taxol: staining was seen only in A2780/C3 tumors (but not in A2780 tumors) treated with taxol (Figure 5). Positive TUNEL staining was also observable in A2780/C3 tumors treated with 6 mg/kg of taxol (data not shown).

Discussion

p53 is an important determinant of cancer-cell susceptibility to drug treatment, although its role as prognostic factor is still controversial. Its activation, mainly through posttranscriptional stabilization, can lead to cell cycle arrest or to apoptosis. The cellular context and the extent and type of damage induced dictate whether cell death or cell cycle arrest will happen [4–6]. In some cell types the cell damage dependent *p53* induction leads mainly to cell cycle arrest [11,19]. This is particularly true for ovarian cancer cells where *p53*-dependent apoptosis is often very limited after drug treatment [19,20] with one of the reasons being the high *p53*-dependent expression of *p21* but not of *bax*. The low *p53*-dependent transcription of *bax* might partly be explained by the imperfect *p53* binding site in the promoter of *bax* [23] and in fact this promoter is less responsive to *p53* than the *p21* promoter (Hardy-Bessard, AC and Soussi, T., personal communication). The present study was aimed at elucidating whether in a human ovarian cancer cell line expressing wt *p53* (A2780) the forced *p53*-dependent expression of *bax* could direct the response toward cell death rather than cell cycle arrest and whether this shift was accompanied by an increased sensitivity to taxol. *bax* plays an important role in determining the sensitivity to drugs in cells overexpressing this protein and, in general, an increase in *bax* levels is associated with a greater induction of cell death after DNA damage [16,17,24–26]. In these systems, however, the high *bax* levels were not a consequence of drug treatment, but were already present in untreated cells. The present study employed a system in which *bax* can only be activated by *p53* as a consequence of damage in the cell, as clearly demonstrated in the SKOV3/B1 cell line not expressing *p53*, where the construct does not respond to drug-induced damage, but fully responds to cotransfection with a wt *p53* expressing plasmid (data not shown).

We selected taxol for two reasons: first because it is one of the most effective drugs in the treatment of ovarian cancer [27], and second because it is particularly sensitive to the presence of wt *p53* and in cells (including A2780) where the induction of *p53*-dependent cell cycle arrest is predominant, an increase in sensitivity to taxol is observed after disruption of *p53* [11,12]. The striking increase in sensitivity to taxol in the A2780 transfected clones strongly supports findings obtained so far and stress the importance of the cell "decision" to arrest cell cycle or to undergo apoptosis in response to damage. That the balance between *p21* and *bax* is decisive for cellular response was indirectly shown in wt *p53*, p21 −/− cells, which were more susceptible to DNA damage *in vitro* or *in vivo* than the corresponding wt *p53*, p21+/+ cells [6,14]. The mechanism by which *bax* promotes apoptosis in these cells is not clear. A release of cytochrome *c* from mitocondria was observed either after short or long taxol treatment in A2780/C3 cells but not in A2780 parental cells (data not shown) and this could be important for the induction of apoptosis and confirmed the *bax*-dependent release of cytochrome *c* from mitochondria previously described in other systems [18,28]. This mechanism, and the consequent plausible activation of caspases, is under study in these cell systems.

The results in nude mice bearing *p53*-dependent, *bax*-transfected tumor support the hypothesis that *p53*-induced apoptosis can indeed increase human tumor sensitivity to chemotherapy. It is worth noting that a significant response of A2780/C3 xenografts was obtained on advanced stage tumors (treatment starting on 300 mg tumors) and at doses of taxol lower than those described as optimal in similar tumor models [29]. The clear demonstration in these cellular systems that the increase in apoptosis is *p53*-dependent and *bax*-mediated supports *bax* as one of the mediators of *p53*-induced apoptosis *in vitro* and *in vivo*, and open up the possibility to study possible modifications of *p53* structure which could preferentially activate *bax* rather than *p21*. This possibility is not remote as changes in phosphorylation of *p53* could indeed influence its transcriptional activity [30–32]. In addition, DNA damage-induced phosphorylation of the *p53* N-terminus has been reported [33,34] and studies in cells with selective alterations of putative kinases responsible for these phosphorylations (DNA-PK, CK, ATM and so on) could help in clarifying whether these posttranslational modifications are also important for *p53* to decide which downstream effector has to be mainly activated. Although at present entirely speculative, it would also be important to understand whether kinase-specific inhibitors/activators could be used as *p53* drivers. Moreover, induction of conformational changes in *p53* mutants (which would allow specific gene transcriptional activation, i.e., *bax* and not *p21*) would be an interesting area of research in which to seek highly selective anticancer drugs.

Acknowledgments

This work was partially supported by the project No. ICS120/RF98/73 of the Italian Ministry of Health and by the "CNR progetto strategico apoptosi No. 96.01026.ST74". The generous contribution of the Italian Association for Cancer

Research (AIRC) and Italian Foundation for Cancer Research (FIRC) is gratefully acknowledged. Paola De Feudis is a ''Famiglie Belloni e Guglielmetti'' fellow.

References

[1] Ko LJ, and Prives C (1996). *p53*: puzzle and paradigm. *Genes Dev* **56**, 2649–2654.

[2] Levine AJ (1997). *p53*, the cellular gatekeeper for growth and division. *Cell* **88**, 323–331.

[3] Oren M, and Prives C (1996). *p53*: upstream, downstream and off stream. Review of the 8th *p53* workshop (Dundee, July 5–9, 1996). *Biochim Biophys Acta* **1288**, R13–R19.

[4] Chen X, Ko LJ, Jayaraman L, and Prives C (1996). *p53* levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev* **10**, 2438–2451.

[5] Bates S, and Vousden KH (1996). *p53* in signaling checkpoint arrest or apoptosis. *Curr Opin Genet Dev* **6**, 12–18.

[6] Waldman T, Zhang Y, Dillehay L, Yu J, Kinzler K, Vogelstein B, and Williams J (1997). Cell-cycle arrest versus cell death in cancer therapy [see comments]. *Nat Med* **3**, 1034–1036.

[7] el Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, and Vogelstein B (1993). WAF1, a potential mediator of *p53* tumor suppression. *Cell* **75**, 817–825.

[8] Harper JW, Adamy GR, Wei N, Keyomarsi K, and Elledge SJ (1993). The *p21* Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* **75**, 805–816.

[9] Oltvai ZN, Milliman CL, and Korsmeyer SJ (1993). Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **74**, 609–619.

[10] Lill NL, Grossman SR, Ginsberg D, DeCaprio J, and Livingston DM (1997). Binding and modulation of *p53* by p300/CBP coactivators. *Nature* **387**, 823–827.

[11] Vikhanskaya F, Vignati S, Beccaglia P, Ottoboni C, Russo P, D'Incalci M, and Broggini M (1998). Inactivation of *p53* in a human ovarian cancer cell line increases the sensitivity to paclitaxel by inducing G2/M arrest and apoptosis. *Exp Cell Res* **241**, 96–101.

[12] Wahl AF, Donaldson KL, Fairchild C, Lee FY, Foster SA, Demers GW, and Galloway DA (1996). Loss of normal *p53* function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. *Nat Med* **2**, 72–79.

[13] Fan S, Smith ML, Rivet DJ, Duba D, Zhan Q, Kohn KW, Fornace AJJ, and O'Connor PM (1995). Disruption of *p53* function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. *Cancer Res* **55**, 1649–1654.

[14] Waldman T, Kinzler KW, and Vogelstein B (1995). *p21* is necessary for the *p53*-mediated G1 arrest in human cancer cells. *Cancer Res* **55**, 5187–5190.

[15] Sakakura C, Sweeney EA, Shirahama T, Igarashi Y, Hakomori S, Nakatani H, Tsujimoto H, Imanishi T, Ohgaki M, Ohyama T, *et al.* (1996). Overexpression of *bax* sensitizes human breast cancer MCF-7 cells to radiation-induced apoptosis. *Int J Cancer* **67**, 101–105.

[16] Yin C, Knudson CM, Korsmeyer SJ, and Van Dyke T (1997). Bax suppresses tumorigenesis and stimulates apoptosis *in vivo*. *Nature* **385**, 637–640.

[17] Strobel T, Swanson L, Korsmeyer S, and Cannistra SA (1996). BAX enhances paclitaxel-induced apoptosis through a *p53*-independent pathway. *Proc Natl Acad Sci USA* **93**, 14094–14099.

[18] Rossè T, Olivier R, Monney L, Rager M, Conus S, Fellay I, Jansen B, and Borner C (1998). Bcl-2 prolongs cell survival after Bax-induced release of cytochrome *c*. *Nature* **391**, 496–499.

[19] Debernardis D, Sire EG, De Feudis P, Vikhanskaya F, Valenti M, Russo P, Parodi S, D'Incalci M, and Broggini M (1997). *p53* status does not affect sensitivity of human ovarian cancer cell lines to paclitaxel. *Cancer Res* **57**, 870–874.

[20] De Feudis P, Debernardis D, Beccaglia P, Valenti M, Graniela Siré EA, Arzani D, Stanzione S, Parodi S, D'Incalci M, Russo P, *et al.* (1997). DDP-induced cytotoxicity is not influenced by *p53* in nine human ovarian cancer cell lines with different *p53* status. *Br J Cancer* **76**, 474–479.

[21] Sambrook J, Fritsch EF, and Maniatis T (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

[22] Boven E, Winograd B, Berger DP, Dumont MP, Braakhuis BJ, Fodstad O, Langdon S, and Fiebig HH (1992). Phase II preclinical drug screening in human tumor xenografts: a first European multicenter collaborative study. *Cancer Res* **52**, 5940–5947.

[23] Miyashita T, and Reed JC (1995). Tumor suppressor *p53* is a direct transcriptional activator of the human *bax* gene. *Cell* **80**, 293–299.

[24] Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius V, Niskanen E, Nordling S, and Reed JC (1995). Reduced expression of proapoptotic gene *bax* is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. *Cancer Res* **55**, 4471–4478.

[25] Simonian PL, Grillot DA, Andrews DW, Leber B, and Nunez G (1996). Bax homodimerization is not required for Bax to accelerate chemotherapy-induced cell death. *J Biol Chem* **271**, 32073–32077.

[26] Kobayashi T, Ruan S, Clodi K, Kliche OK, Shiku H, Andreef M, and Zhang W (1998). Overexpression of *bax* gene sensitizes K562 erythroleukemia cells to apoptosis induced by selective chemotherapeutic agents. *Oncogene* **16**, 1587–1591.

[27] Rowinsky EK, and Donehower RC (1995). Paclitaxel (taxol). *N Engl J Med* **332**, 1004–1014.

[28] Yang J, Liu X, Bhalla K, Kim N, Ibrado AM, Cai J, Peng T, Jones DP, and Wang X (1997). Prevention of apoptosis by bcl-2: release of cytochrome *c* from mitochondria blocked. *Science* **275**, 1129–1132.

[29] Nicoletti MI, Lucchini V, Masazza G, Abbott BJ, D'Incalci M, and Giavazzi R (1993). Antitumor activity of taxol (NSC-125973) in human ovarian carcinomas growing in the peritoneal cavity of nude mice. *Ann Oncol* **4**, 151–155.

[30] Adler V, Pincus MR, Minamoto T, Fuchs SY, Bluth MJ, Brandt Rauf PW, Friedman FK, Robinson RC, Chen JM, Wang XW, *et al.* (1997). Conformation-dependent phosphorylation of *p53*. *Proc Natl Acad Sci USA* **94**, 1686–1691.

[31] Reinke V, and Lozano G (1997). Differential activation of *p53* targets in cells treated with ultraviolet radiation that undergo both apoptosis and growth arrest. *Radiat Res* **148**, 115–122.

[32] Lohrum M, and Scheidtmann KH (1996). Differential effects of phosphorylation of rat *p53* on transactivation of promoters derived from different *p53* responsive genes. *Oncogene* **13**, 2527–2539.

[33] Siciliano JD, Canman CE, Taya Y, Sakaguchi K, Appella E, and Kastan MB (1997). DNA damage induces phosphorylation of the amino terminus of *p53*. *Genes Dev* **11**, 3471–3481.

[34] Shieh S-Y, Ikeda M, Taya Y, and Prives C (1997). DNA damage-induced phosphorylation of *p53* alleviates inhibition by MDM2. *Cell* **91**, 325–334.