p53-Dependent Apoptosis in Melanoma Cells After Treatment with Camptothecin[1]

Gang Li, Jason A. Bush, and Vincent C. Ho

Department of Medicine, Division of Dermatology, Vancouver Hospital and Health Sciences Center, The University of British Columbia, Vancouver, British Columbia, Canada

Cutaneous malignant melanoma is a life-threatening cancer with poor prognosis due to a high metastasis potential. The main obstacle in treatment of metastatic melanoma is the resistance to chemotherapy. Recent studies indicated that apoptosis is a common mechanism of action for various cytotoxic agents. As p53 plays an important part in apoptosis, we investigated the role of p53 in chemosensitivity of melanoma cells. Previously, we found that melanoma cell lines containing wild-type p53 have significantly higher response rates to chemotherapy than cell lines with a mutant p53 gene. To confirm the role of p53 in melanoma chemosensitivity further, we transfected an expression vector, pED1, which carries a mutant p53 gene, into a wild-type p53 melanoma cell line, MMAN. We examined the effect of mutant p53 on camptothecin-induced apoptosis and the expression of genes which are known to be involved in apoptosis or drug resistance, such as bcl-2, bax, bak, $p21^{waf1}$, and P-glycoprotein. Our results indicate that overexpression of the mutant p53 increased the growth rate of MMAN cells, reduced the sensitivity to camptothecin, and lowered drug-induced apoptosis by 2–3-fold. Flow cytometry indicated that the camptothecin-induced apoptosis is not associated with G_1 arrest. Furthermore, camptothecin treatment reduced bcl-2 and P-glycoprotein expression in wild-type p53 MMAN cells, but not cells overexpressing mutant p53. These results demonstrate that p53 mutational status is a determinant of melanoma chemosensitivity. p53 may downregulate bcl-2 and P-glycoprotein to induce apoptosis in melanoma cells after chemotherapy. ***Key words: bcl-2/chemotherapy/drug resistance/melanocytes. J Invest Dermatol 114:514–519, 2000***

P53 is one of the most frequently mutated genes in human cancers. Overexpression of the p53 tumor suppressor is frequently observed in metastatic melanoma (MM) but not in primary melanoma (Cristofolini *et al*, 1993; Lassam *et al*, 1993; Li *et al*, 1995; Hartmann *et al*, 1996). DNA sequencing studies indicated that mutation of the p53 gene occurs in 25–30% of metastatic melanomas, but not in benign nevi, suggesting that mutation of the p53 gene may play a part in the progression of MM (Sparrow *et al*, 1995; Hartmann *et al*, 1996). To support this hypothesis, recent studies have shown that p53 overexpression is associated with increased depth of invasion of melanoma (both Breslow thickness and Clark's levels), tumor ulceration, and poor prognosis (Korabiowska *et al*, 1995; Weiss *et al*, 1995; Yamamoto *et al*, 1995).

MM is a life-threatening skin cancer which metastasizes very rapidly. Metastatic MM is usually incurable, with a 5 y survival < 10% and a poor response to chemotherapy (Koh, 1991; Roses *et al*, 1991). The reason for the relative resistance of metastatic MM to anti-cancer drugs is unknown. Recently, accumulated evidence has suggested that anti-cancer drugs exert their cytotoxicity predominantly by inducing apoptosis in tumor cells (Kamesaki, 1998; Sekiguchi *et al*, 1998). Therefore, dysregulation of the genes involved in the process of apoptosis may contribute to the chemoresistance.

p53 plays a crucial part in apoptosis. Wild-type p53 function is required for apoptosis of cultured cells induced by ionizing radiation (Lowe *et al*, 1993), UV irradiation (Tron *et al*, 1998), as well as anti-cancer drugs (Li *et al*, 1998; Miyake *et al*, 1998; Petty *et al*, 1998). p53 controls apoptosis most likely through the transcriptional regulation of target genes, such as bax, and bcl-2. Bcl-2 protein is thought to prevent most types of apoptotic cell death, whereas bax protein (a homologous protein to bcl-2) heterodimerizes with bcl-2 and promotes apoptosis (Oltvai *et al*, 1993). Previous studies of non-Hodgkin's lymphomas and breast cancers have demonstrated an inverse relationship between p53 mutation and bcl-2 expression (Pezzella *et al*, 1993). p53 protein has been found to be a regulator of bcl-2 and bax expression both *in vitro* and *in vivo* (Miyashita *et al*, 1994). The dysregulation of bax/bcl-2 by mutant p53 protein may lead to changes in the apoptotic rate in melanoma after chemotherapy.

We have previously compared the response rate to anti-cancer drugs of the melanoma cell lines which have different p53 status. We found that melanoma cell lines which carry a wild-type p53 gene have much higher response rates to chemotherapy than cell lines with a mutant p53 gene. To confirm the role of p53 in melanoma chemosensitivity further, we transfected a mutant p53 gene into a melanoma cell line, MMAN, which carries a wild-type p53 gene. We treated the cells with the anti-cancer drug,

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Reprint requests to: Dr. Gang Li, Jack Bell Research Center, 2660 Oak Street, Vancouver, BC, Canada V6H 3Z6. Email: gangli@unixg.ubc.ca

Abbreviations: CPT, camptothecin; ELISA, enzyme-linked immunosorbent assay; mdr, multidrug resistance gene; MM, melanoma; Pgp, P-glycoprotein; SRB, sulforhodamine.

[1]The authors declared in writing to have no conflict of interest.

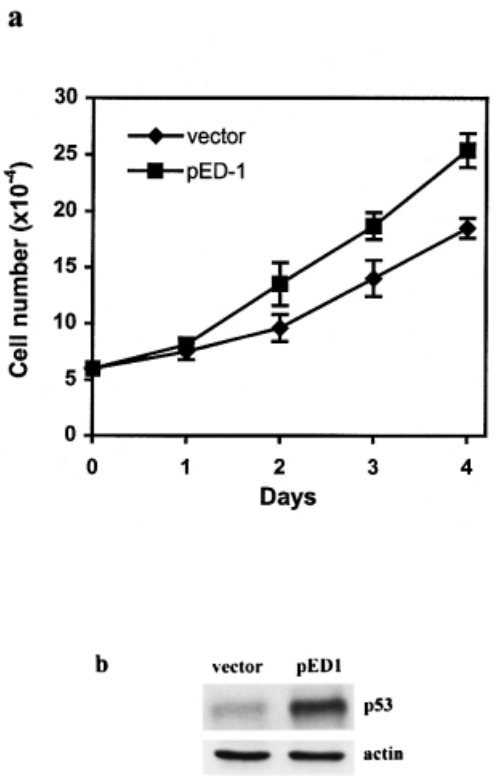

Figure 1. Overexpression of mutant p53 on growth rate of the melanoma cell line MMAN. (*a*) MMAN cells containing either pED1 or vector alone were seeded at 6×10^4 cells/35 mm Petri dishes, and cultured for 4 d. The cells were counted with a hemocytometer at 24 h intervals. (*b*) p53 level in MMAN-pED1 and MMAN-vector cells by Western blotting. CM1 antibody detects both wild-type and mutant p53.

camptothecin, a DNA-damaging topoisomerase I inhibitor, and then compared the apoptosis rate and the expression of apoptotic genes between the MMAN cell lines carrying either wild-type or mutant p53. Here we report that overexpression of mutant p53 reduces CPT-induced apoptosis in a melanoma cell line, possibly by interfering with the wild-type p53 downregulation of bcl-2 and P-glycoprotein (Pgp).

MATERIALS AND METHODS

Cell culture The melanoma cell line MMAN was a kind gift from Dr. R. Byers, Boston University School of Medicine (Boston, MA) (Byers *et al*, 1991). The cells were grown at 37°C in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, 100 units penicillin G per ml and 100 μg streptomycin per ml in an atmosphere containing 5% (vol/vol) CO_2.

Reagents Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin G, streptomycin, G418, proteinase K, and agarose were obtained from Canadian Life Technologies (Mississauga, Ontario, Canada). CPT and RNase were obtained from Sigma (Mississauga, Ontario, Canada). Stock solutions with CPT (10 mM) were prepared in dimethylsulfoxide and stored at –20°C.

Expression of mutant p53 in MMAN cell line The eukaryotic expression vector pED1, which contains a point mutation in the human p53 cDNA that changes Cys-135 to serine, was a kind gift from Dr. S. Benchimol (University of Toronto, Canada). The melanoma cell line MMAN was transfected with the pED1 plasmid DNA or vector DNA by lipofection (Li *et al*, 1996). The transfectants were selected with G418 (400 μg per ml) for 3 wk.

Cytotoxicity assay Cells were plated on to 96-well microtitre plates at 1×10^4 per well, grown for 24 h and treated with different concentrations of anti-cancer drugs for 48 h. Relative toxicity was determined by the sulforhodamine (SRB) assay as described elsewhere (Skehan *et al*, 1990; Li *et al*, 1998). SRB is a bright pink aminoxanthene dye with two sulfonic

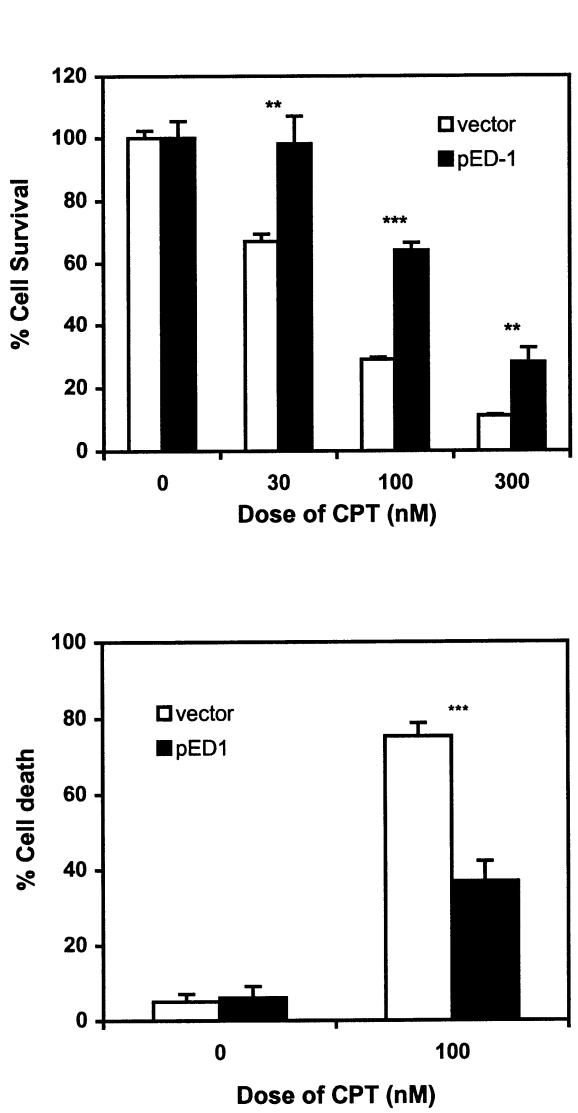

Figure 2. Overexpression of mutant p53 on the sensitivity of MMAN cells to CPT treatment. (*a*) SRB assay. Cells were seeded on to 96-well plates at 1×10^4 per well for 24 h, treated with 0, 30, 100, or 300 nM of CPT for 48 h. The survival rate was determined by SRB assay (see *Materials and Methods*). (*b*) Trypan blue expression assay. Cells were seeded on to 60 mm dishes at 5×10^5 cells per dish for 24 h, and treated with 100 nM CPT for 48 h. The floating and attached cells were pooled and resuspended in 0.4% Trypan blue/PBS. Both viable and dead cells were counted with a hemocytometer.

groups. Under mildly acidic conditions, SRB binds to protein basic amino acid residues in TCA-fixed cells to provide a sensitive index of cellular protein content. Briefly, the cells were fixed with 10% trichloroacetic acid for 1 h at 4°C, rinsed five times with water, and air dried. The cells were then fixed with 0.4% SRB in 1% acetic acid for 30 min. After rinsing four times with 1% acetic acid and air dried, 100 μl of 10 mM Tris (pH 10.5) was added to the wells for 30 min. The colorimetric reading was carried out in a Microplate Autoreader (Bio-Tek Instruments, Winooski, VT) at 550 nm.

Enzyme-linked immunosorbent assay (ELISA) of apoptosis Cells were seeded on to 96-well microtitre plates at a density of 2×10^4 per well, grown for 24 h, and exposed to anti-cancer drugs for 24 h. The microtitre plates were centrifuged at $2000 \times g$ for 10 min. The ELISA was performed using a Cell Death Detection Elisa^{plus} kit (Boehringer Mannheim, Quebec, Canada) according to the manufacturer's protocol. Briefly, the cells were resuspended in 200 μl lysis buffer and incubated for 30 min at room temperature. The lysate was centrifuged at $2000 \times g$ for 10 min and 150 μl of supernatant was collected. Then 20 μl of the supernatant was incubated with antihistone-biotin and anti-DNA peroxidase at room temperature for

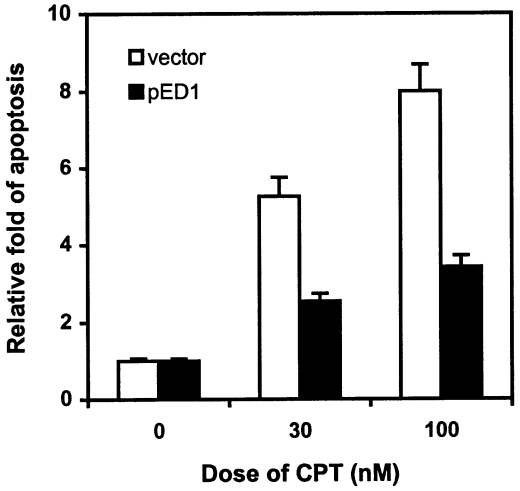

Figure 3. Overexpression of mutant p53 on CPT-induced apoptosis. Cells were seeded on to a 96-well plate at 2×10^4 per well, treated with 30 or 100 nM CPT for 24 h, and the apoptosis rate was determined by colorimetric analysis using a Cell Death Detection ELISA^{plus} kit (see *Materials and Methods*).

2 h. After washing the wells three times with incubation buffer, 100 µl of substrate solution (2,2′-azino-bis[3-ethylbenzthiazoline-sulfonic acid]) was added to each well for 15–30 min. The colorimetric analysis was carried out in a Microplate Autoreader at 405 nm.

Flow cytometry Confluent cells ($\approx 1 \times 10^6$) were harvested by trypsinization, neutralized in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum, then washed twice in cold phosphate-buffered saline (PBS)/5 mM ethylenediamine tetraacetic acid. Cell pellets were resuspended after centrifugation ($1000 \times g$ for 3 min) in 250 µl cold PBS/5 mM ethylenediamine tetraacetic acid followed by slow addition of 750 µl cold 100% ethanol. Cells were mixed well and fixed for a minimum of 1 h at 4°C. After fixation, cells were washed twice in cold PBS then stained in the dark with 1 ml PBS containing 50 µg per ml propidium iodide (Sigma) and 20 µg per ml RNaseA (Gibco) for 20 min at 37°C. Cell suspensions were then run on a Coulter Epics Elite flow cytometer (Coulter) and analyzed with multicycle (Phoenix software).

Gel electrophoresis Cells were seeded at a density of 2×10^6/100 mm dishes for 24 h, and exposed to CPT for 24 h. Both the detached and the attached cells were collected and pelleted by centrifugation. The cells were incubated in 500 µl of a lysis buffer containing 100 mM NaCl, 20 mM ethylenediamine tetraacetic acid, 50 mM Tris–Cl (pH 8.0), 0.5% sodium dodecyl sulfate and 1 mg proteinase K per ml at 50°C for 3 h. DNA was extracted with phenol–chloroform and ethanol precipitation. RNA was removed by digestion with pancreatic RNase. The DNA was analyzed on 2% agarose gel for DNA fragmentation. The gels were stained with 0.5 µg ethidium bromide per ml and photographed under ultraviolet light with a Gel Print System (Biophotonics, Ann Arbor, MI). The intensity of the bands were quantified with a phosphoimager (Bio-Rad, Ontario, Canada).

Western blot analysis Cells were washed with PBS three times, and lyzed in triple-detergent buffer [50 mM Tris–Cl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 0.1% sodium dodecyl sulfate, 100 µg phenylmethylsulfonyl fluoride per ml, 1 µg aprotinin per ml, 1% Nonidet p-40, 0.5% sodium deoxycholate] for 30 min. The lysate was centrifuged at $12{,}000 \times g$ for 10 min and the supernatant collected. The protein concentration was determined by the DC Protein Assay (Bio-Rad). One hundred micrograms of protein was separated on a 12% polyacrylamide/sodium dodecyl sulfate gel, and electroblotted on to a nitrocellulose filter. The filter was blocked with 5% milk overnight, hybridized to anti-p53 CM1 antibody (Dimension, Mississauga, Ont), anti-p21^{waf1} antibody (Santa Cruz, CA), or anti-bax antibody (Santa Cruz, CA) for 1 h, and then hybridized with horseradish peroxidase-labeled secondary antibody for 1 h at room temperature. The signals were detected with ECL-western chemiluminescence detection kit (Amersham, Quebec, Canada).

RESULTS

Expression of mutant p53 gene increases the growth rate of the melanoma cell line MMAN The melanoma cell line MMAN contains a wild-type p53 gene (Li *et al*, 1995). Previously, we have shown that four wild-type p53 melanoma cell lines have higher response rates to chemotherapy than four mutant p53 melanoma cell lines (Li *et al*, 1998). To confirm further that the difference in chemosensitivity between wild-type p53 and mutant p53 melanoma cell lines is due to wild-type p53-induced apoptosis, we transfected the wild-type p53 melanoma cell line MMAN with an expression vector carrying a mutant p53 gene. After selection with G418, the transfectants which carry mutant p53 genes were expanded into a cell line, designated as MMAN-pED1. MMAN cells transfected with vector alone were established as controls. Western blot analysis using a polyclonal p53 antibody CM1, which recognizes both wild-type and mutant p53, showed that MMAN-pED1 cells have higher levels of p53 protein compared with MMAN alone, indicating that the mutant p53 gene is expressed in the transfectants (**Fig 1**).

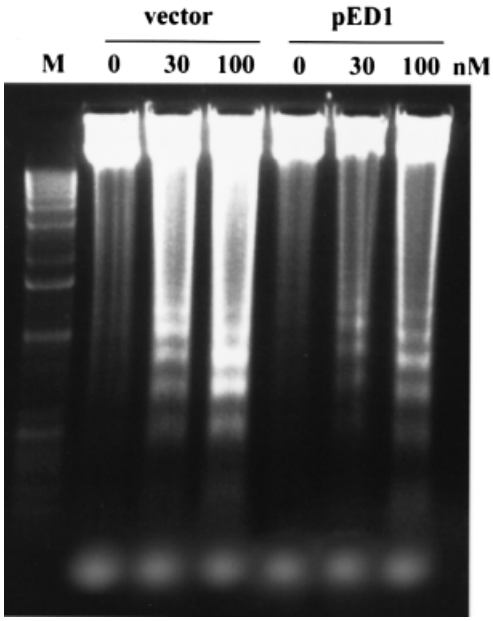

Figure 4. Overexpression of mutant p53 on CPT-induced DNA fragmentation. Cells were seeded at a density of 2×10^6/100 mm dishes for 24 h, and exposed to 0, 30, or 100 nM of CPT for 24 h. The DNA were extracted from the cells and electrophoresized on a 2% agarose gel.

To investigate if overexpression of the mutant p53 protein has any effect on cell growth, we compared the growth rate of MMAN-pED1 and MMAN-vector cells. **Figure 1** shows that the growth rate of MMAN-pED1 cells increased by 37% compared with MMAN-vector cells after a 4 d culture ($p < 0.01$, t test).

CPT-induced apoptosis is p53 dependent We treated the MMAN-pED1 and MMAN-vector cells with the anti-cancer drug CPT and compared the chemosensitivity between these two cell lines. SRB cytotoxicity assay indicates that cells overexpressing mutant p53 are significantly more resistant to CPT (**Fig 2*a***). After treatment with 30 nM, 100 nM, or 300 nM of CPT for 48 h, the survival rates of MMAN-pED1 cells were 98%, 64%, and 28%, compared with 67%, 29%, and 11% in the controls ($p = 0.004$, 0.00002, and 0.004, respectively, t test). The IC50 is increased by 3-fold in MMAN-pED1 cells compared with that of MMAN-vector cells (180 nM *vs* 60 nM). To confirm the results of SRB assay, we used Trypan blue exclusion to determine the percentage of dead cells after CPT treatment in these two cell types. **Figure 2(*b*)** demonstrates that CPT induced significantly more cell death in MMAN than pED1 cells ($p = 0.0005$, t test).

To investigate whether the resistance of MMAN-pED1 cells to chemotherapy is due to a reduced apoptotic rate, we used ELISA and DNA fragmentation assays to examine the anti-cancer drug-

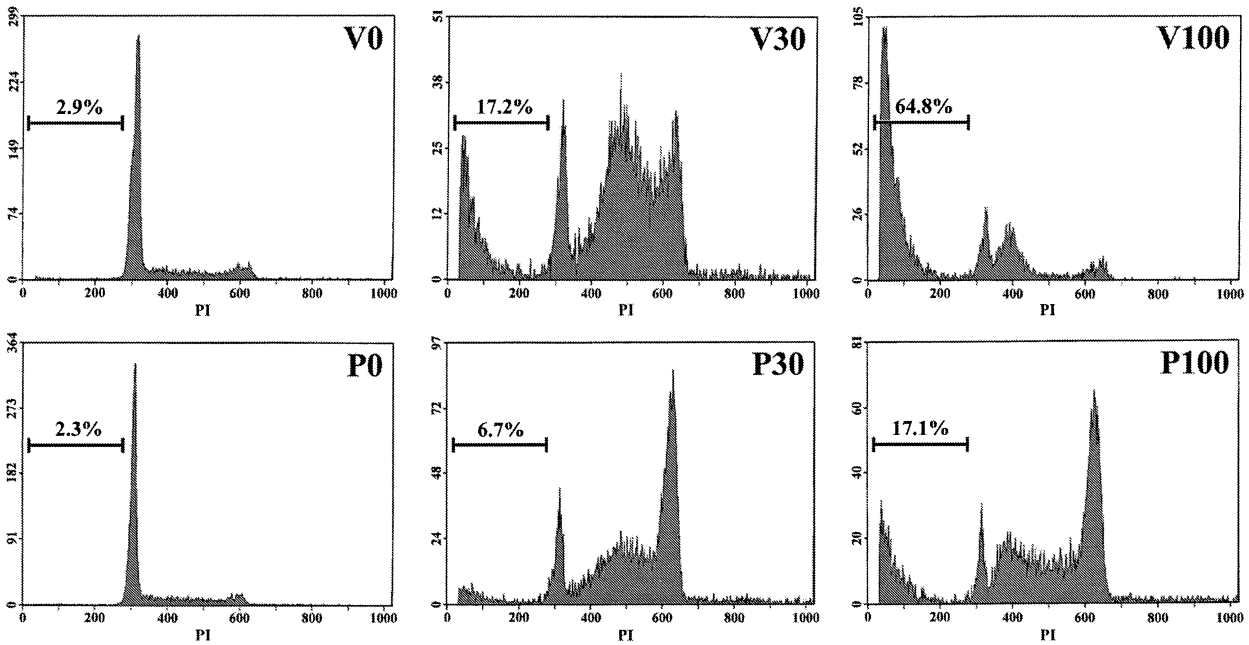

Figure 5. Effect of mutant p53 on CPT-induced apoptosis by flow cytometry. Cells were treated with CPT for 24 h and analyzed by flow cytometry as described in *Materials and Methods*. The percentage of the apoptotic cells with hypodiploid DNA content is shown by the brackets over the sub-G_0/G_1 peak. Cell number is shown on the y-axis. PI, propidium iodide. V0, V30, V100: MMAN-vector cells treated with CPT at 0, 30, or 100 nM. P0, P30, P100: MMAN-pED1 cells treated with CPT at 0, 30, or 100 nM.

induced apoptosis in MMAN-pED1 and MMAN-vector cells. **Figure 3** shows that the apoptosis rate was reduced by 52% and 57% in MMAN-pED1 cells at 30 nM and 100 nM, respectively ($p = 0.005$, t test). To confirm further that anti-cancer drug-induced apoptosis is reduced in MMAN-pED1 cells, MMAN-vector and MMAN-pED1 cells were treated with 0, 30, and 100 nM of CPT for 24 h. Attached and floating cells were pooled and the DNA samples were analyzed by gel electrophoresis. **Figure 4** demonstrates that DNA fragmentation is reduced by 3.5-fold in MMAN-pED1 cells (phosphoimager scanning). In addition, we performed flow cytometry to determine if mutant p53 renders resistance to CPT-induced apoptosis. **Figure 5** demonstrates that mutant p53-containing MMAN-pED1 cells have significantly less apoptosis than MMAN-vector cells. At 30 nM and 100 nM, the apoptosis rate is 2.5- and 3.8-fold lower in pED1 cells compared with MMAN-vector cells. To establish the relationship between CPT-induced apoptosis and cell cycle arrest, we performed cell cycle analysis on CPT-treated MMAN-vector and MMAN-pED1 cells. **Table I** demonstrates that CPT induced S/G_2 arrest in both MMAN-vector and MMAN-pED1 cells, indicating that the S/G_2 arrest is p53-independent.

Reduction of bcl-2 is p53-dependent after CPT treatment p53 is a transcriptional regulator. To investigate what genes are regulated by p53 after anti-cancer drug treatment, we examined the expression of $p21^{waf1}$, bax, bcl-2, bak, and Pgp in MMAN-vector and MMAN-pED1 cells before and after 100 nM of CPT treatment. p53 expression was dramatically increased after CPT treatment. The expression of bcl-2 and Pgp was reduced in MMAN-vector but not in MMAN-pED1 cells after CPT treatment, whereas the expression of bax, bak, and $p21^{waf1}$ did not significantly change (**Fig 6**).

DISCUSSION

Melanoma is a life-threatening skin cancer which has high potential for metastasis. Unfortunately, there is still no cure for metastatic melanoma. One major obstacle in melanoma treatment is the resistance of metastatic melanoma to chemotherapy. Many studies have indicated that mutation of the p53 gene is associated with increased depth of invasion, low response rate of chemotherapy, and poor prognosis in MM (Korabiowska *et al*, 1995; Weiss *et al*, 1995; Yamamoto *et al*, 1995). Previously, we have demonstrated that melanoma cell lines carrying a wild-type p53 gene have greater response rates to various types of anti-cancer drugs than mutant p53 melanoma cell lines (Li *et al*, 1998). The objective of this study was to investigate further the importance of the p53 function in chemotherapy-induced apoptosis of melanoma cells. We chose CPT as the chemotherapeutic agent in this study because CPT is known to induce apoptosis in various types of tumor cells and CPT has shown potential in clinical trials (Pantazis, 1995; Muggia *et al*, 1996; Stewart *et al*, 1996; Rothenberg, 1997). In addition, CPT demonstrated a broad spectrum of anti-tumor activity due to the lack of clinical cross-resistance with existing anti-neoplastic compounds (Rothenberg, 1997). Our results indicate that over-expression of a mutant p53 gene increased the growth rate of MMAN cells (**Fig 1**), reduced the response rate to chemotherapy (**Fig 2**), and reduced the apoptosis rate after CPT treatment (**Figs 3–5**). Reduced chemosensitivity was also observed in mutant p53 cells after treatment with another anti-cancer drug vincristine (data not shown). Furthermore, overexpression of a dominant negative mutant p53 abolished the downregulation of bcl-2 and Pgp in response to CPT treatment (**Fig 6**).

It is known that p53 upregulates $p21^{waf1}$, which is a cyclin-dependent kinase inhibitor, and therefore arrests cells in the G_1 phase of the cell cycle (Haffner and Oren, 1995). There is, however, no significant change in $p21^{waf1}$ expression between wild-type and mutant p53 cells before or after CPT treatment (**Fig 6**), indicating that CPT-induced cell death is not related to p53-induced G_1 arrest. Cell cycle analysis clearly demonstrates that CPT induced a p53-independent S/G_2 phase arrest, not G_1 arrest (**Table I**). Our data support the findings that: (i) CPT induces break points in late S and G_2 phases (Bassi *et al*, 1998); (ii) increased sensitivity to CPT is not associated with the loss of a G_1 checkpoint (Slichenmyer *et al*, 1993); and (iii) CPT treatment results in an accumulation of cells in the G_2/M phase (Jones *et al*, 1997).

Recent research has indicated that apoptosis is the primary mechanism of the cytotoxicity for various types of anti-cancer drugs (Kamesaki, 1998; Sekiguchi *et al*, 1998). Therefore, the expression of the apoptotic regulators may influence the outcome of chemotherapy. It is well known that p53 regulates the process of

Table I. Cell cycle analysis of CPT-treated MMAN-vector and MMAN-PED1 cells by flow cytometry[a]

Cell type	CPT dose (nM)	% G1	% S	% G2/M
MMAN-vector	0	69.3 ± 4.2	26.1 ± 3.0	4.6 ± 0.9
	30	14.2 ± 1.1	57.5 ± 5.6	28.3 ± 3.1
	100	10.7 ± 1.3	40.9 ± 3.5	48.4 ± 5.0
MMAN-pED1	0	68.8 ± 5.6	29.0 ± 2.8	2.2 ± 0.3
	30	10 ± 0.9	44.8 ± 4.1	45.2 ± 5.2
	100	6.1 ± 0.8	55.6 ± 4.7	38.3 ± 3.6

[a]Cells were treated with CPT for 24 h. Data represent mean ± SD from triplicates.

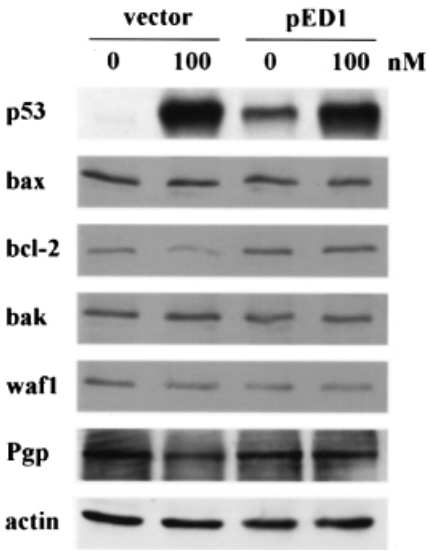

Figure 6. The effect of mutant p53 on bax, bcl-2, p21^{waf1}, and Pgp protein expression. MMAN-vector and MMAN-pED1 cells were treated with or without 100 nM CPT for 24 h. Proteins were extracted and subjected to western blot analysis.

apoptosis. Our finding that a melanoma cell line overexpressing a mutant p53 gene has a lower rate of apoptosis supports the notion that mutant p53 binds and inactivates the wild-type p53 protein and impairs the apoptotic process. The exact mechanisms as to how p53 triggers apoptosis in melanoma cells after chemotherapy is unknown, it appears that p53 downregulates bcl-2 in melanoma cells to trigger apoptosis.

Bcl-2 and bax are a pair of pivotal genes that control programmed cell death, or apoptosis, with bax being the apoptosis promoter and bcl-2 the apoptosis inhibitor. Bax and bcl-2 form protein dimers with each other and the relative ratio of bax/bcl-2 is believed to be a determinant of the balance between life and death within a cell (Oltvai *et al*, 1993). Studies indicate that unbalanced bax/bcl-2 expression alters the responses of tumor cells to chemotherapy. For instance, elevated bcl-2/bax is a consistent feature of apoptotic resistance in B cell chronic lymphocytic leukemia and is correlated with *in vivo* chemoresistance, with the most resistant cells expressing elevated bcl-2 levels and lower bax protein (Pepper *et al*, 1998). Conversely, overexpression of bax gene sensitizes K562 erythroleukemia cells to apoptosis induced by selective chemotherapeutic agents (Kobayashi *et al*, 1998) whereas overexpression of bcl-2 resulted in protection from drug-induced apoptosis of ovarian carcinoma cells (Eliopoulos *et al*, 1995).

Some studies indicate that p53 may be the upstream regulator of bax/bcl-2. Bcl-2 overexpression and bax downregulation was associated with p53 mutant immunophenotype in neuroendocrine lung tumors (Brambilla *et al*, 1996). The expression of bcl-2 is p53-dependent in acute lymphoblastic leukemia, and in most wild-type p53 cells ionizing radiation induces downregulation of bcl-2 and upregulation of bax (Findley *et al*, 1997). Other studies, however, demonstrate that p53 does not regulate bcl-2 or bax expression. In UV-induced apoptotic mouse keratinocytes, the expression of bcl-2 and bax is independent of p53 status (Li *et al*, 1996; Tron *et al*, 1998). In interleukin-7 treated pro-T apoptotic cells, increased bcl-2 and decreased bax expression were also p53-independent (Kim *et al*, 1998). Therefore, it appears that bax/bcl-2 regulation by p53 is tissue specific and stimulus specific. We observed a reduced bcl-2 expression associated with increased p53 in MMAN-vector cells, indicating that wild-type p53 downregulates bcl-2 and eliminates its apoptosis-suppressing effect. Overexpression of mutant p53 did not downregulate bcl-2 (**Fig 6**) and resulted in protection from drug-induced apoptosis (**Fig 3**). This result agrees with the observations by Eliopoulos *et al* (1995) that overexpression of both bcl-2 and mutant p53 protected the ovarian cancer cells from drug-induced apoptosis and by Jansen *et al* (1998) that bcl-2 anti-sense oligonucleotide treatment enhances the chemosensitivity of human melanoma grown in severe combined immunodeficient mice.

Another factor which may influence the outcome of chemotherapy is the expression level of Pgp. Pgp is the product of the multidrug resistance gene (mdr) family and is associated with the emergence of the mdr phenotype in animal cells (Ling, 1997). Although it is known that the expression of Pgp causes drug resistance, little is known about the regulation of Pgp. Recent work suggests that p53 may regulate the expression of mdr. Overexpression of the mdr1 gene correlates with mutant p53 expression in human non-small cell lung cancer (Galimberti *et al*, 1998), breast cancer (Linn *et al*, 1996), and colorectal cancer metastases (de Kant *et al*, 1996). Overexpression of a *trans*-dominant negative p53 into rodent H35 hepatoma cells produced markedly elevated levels of Pgp and mdr1a mRNA (Thottassery *et al*, 1997). Whereas these studies suggest that mutant p53 upregulates Pgp expression, others indicate that wild-type p53 also upregulates the promoter function and the endogenous expression of the rat mdr1b (Zhou and Kuo, 1998). In addition, a p53 consensus binding site has been identified in the Pgp promoter region (Zhou and Kuo, 1998). These conflicting reports imply a complex interaction between the p53 and mdr genes. Our results demonstrate that upon exposure to CPT, a reduction of Pgp expression is associated with increased wild-type p53, but not mutant p53 expression, implying a downregulation of Pgp by p53. The reduction of Pgp in wild-type p53 cells agrees with our observation that anti-cancer drugs induced more apoptosis in the wild-type p53 cells (**Figs 3–5**). Unchanged Pgp expression in MMAN-pED1 cells suggests that the mutant p53 protein itself is unable to bind to the Pgp promoter, but may interfere with the binding of the wild-type p53 to the Pgp promoter.

Taken together, our results indicate that p53 mutational status is a determinant of melanoma chemosensitivity. p53 may down-regulate both the apoptotic protector bcl-2 and the mdr gene to trigger apoptosis in melanoma cells after exposure to anti-cancer drugs.

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