DNA Damage Increases Sensitivity to Vinca Alkaloids and Decreases Sensitivity to Taxanes through p53-dependent Repression of Microtubule-associated Protein 4

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INTRODUCTION

Treatment of cancer is often empirical, based more on clinical findings and histological appearance of the tumor than on an understanding of the molecular mechanism(s) of drug sensitivity. For example, taxanes, such as paclitaxel and docetaxel, and Vinca alkaloids, such as vinblastine and vincristine, are among the most active drugs in the treatment of breast, lung, and other cancers. Yet only 30–50% of previously untreated patients respond to these individual agents (1).

ABSTRACT

Taxanes and Vinca alkaloids are among the most active classes of drugs in the treatment of cancer. Yet, fewer than 50% of previously untreated patients respond, and clinicians have few ways of predicting who will benefit from treatment and who will not. Mutations in p53 occur in more than half of human malignancies and may alter the sensitivity to a variety of anticancer therapies. We have shown that the transcriptional status of p53 determines the sensitivity to antimicrotubule drugs and that this is mediated through the regulation of microtubule-associated protein 4 (MAP4). Expression of MAP4 is transcriptionally repressed by wild-type p53. Increased expression of MAP4, which occurs when p53 is transcriptionally inactive, increases microtubule polymerization, paclitaxel binding, and sensitivity to paclitaxel, a drug that stabilizes polymerized microtubules. In contrast, overexpression of MAP4 decreases microtubule binding and sensitivity to Vinca alkaloids, which promotes microtubule depolymerization. To determine whether induction of endogenous wild-type p53 by DNA-damaging agents alters the expression of MAP4 and changes the sensitivity to antimicrotubule drugs, we assayed cell lines with wild-type or mutant p53 for the expression of MAP4 and drug sensitivity before and after DNA damage. UV irradiation, bleomycin, and doxorubicin increased wild-type p53 expression and decreased MAP4 expression. These changes were associated with decreased sensitivity to paclitaxel and increased sensitivity to vinblastine. These changes in drug sensitivity were no longer observed when p53 and MAP4 returned to baseline levels. Changes in drug sensitivity following DNA-damaging agents were associated with decreased binding of paclitaxel and increased binding of vinblastine. In contrast, DNA damage did not alter the sensitivity to non-microtubule-active drugs, such as 1β-D-arabinofuranosylcytosine and doxorubicin. Changes in drug sensitivity following DNA-damaging drugs were not observed in cells with mutant p53. These studies demonstrate that induction of wild-type p53 by DNA-damaging agents can affect the sensitivity to antimicrotubule drugs through the regulation of MAP4 expression and may have implications for the design of clinical anticancer therapies.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. C127 cells are mouse ductal carcinoma cells that are transformed by BPV-4 (7); products of BPV-1 gene expression do not bind or promote the degradation of p53. C127 cells were maintained at 37°C in monolayer in DMEM supplemented with 10% fetal bovine serum, 50 units/ml penicillin, and 50 μg/ml streptomycin. The BRK p53-An1 cell line has been described previously (8). Briefly, despite this, clinicians have few ways of predicting who will respond and who will not. As a result, many patients are exposed to highly toxic drugs and suffer the side effects without reaping the benefits.

Vinca alkaloids, which are among the most active classes of drugs in the treatment of cancer, have been shown to stabilize microtubules. However, less than 50% of patients respond to these drugs. This suggests that other factors, such as the expression of microtubule-associated protein 4 (MAP4), may affect drug sensitivity.

Recent studies have shown that the transcriptional status of p53 determines the sensitivity to antimicrotubule drugs. Therefore, we sought to determine whether induction of endogenous wild-type p53 by DNA-damaging agents alters the expression of MAP4 and changes the sensitivity to antimicrotubule drugs. We used cell lines with wild-type or mutant p53 and measured changes in drug sensitivity following DNA damage.

Changes in drug sensitivity following DNA-damaging agents were associated with decreased binding of paclitaxel and increased binding of vinblastine. These changes were no longer observed when p53 and MAP4 returned to baseline levels. Changes in drug sensitivity following DNA-damaging drugs were not observed in cells with mutant p53. These studies demonstrate that induction of wild-type p53 by DNA-damaging agents can affect the sensitivity to antimicrotubule drugs through the regulation of MAP4 expression and may have implications for the design of clinical anticancer therapies.
plasmid pmsvcgval135 was introduced into primary BRK epithelial cells with the adenovirus E1A gene to generate p53-An1 (8). These cells were transfected with the murine tsp53(Val135), a temperature-sensitive p53 protein that assumes the mutant conformation at the restrictive temperature (38.5°C) but is predominantly in the wild-type conformation at the permissive temperature (32°C; Ref. 8). BRK cells were maintained at 38.5°C and grown in monolayer in DMEM supplemented with 10% fetal bovine serum, 50 units/ml penicillin, and 50 μg/ml streptomycin.

LnCAP cells and PA-1 cells were obtained from American Type Culture Collection (Manassas, VA). LnCAP is a human prostate cancer cell line that has been described previously (9, 10), and it carries wild-type alleles of p53 (11). LnCAP cells were maintained in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) with 2 mM L-glutamine adjusted to contain 1.5 g/liter sodium bicarbonate, 4.5 g/liter glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, and 10% fetal bovine serum. PA-1 is a human ovarian carcinoma cell line (12, 13) with wild-type p53 (14, 15). PA-1 cells were maintained in monolayer in DMEM supplemented with 10% fetal bovine serum, 50 units/ml penicillin, and 50 μg/ml streptomycin.

All cell lines were grown in an atmosphere of 95% air-5% CO₂, routinely checked, and found to be free of contamination by *Mycoplasma* and fungi.

UV Irradiation. UV irradiation was administered as described previously (4) using a dose of 10 J/m² at 254 nm with a germicidal lamp (Fisher Scientific, Springfield, NJ). This dose had minimal effect upon the growth and distribution of these cells.

Drugs. Bleomycin and doxorubicin were obtained from Bristol-Myers Squibb (Princeton, NJ) and Sigma Chemical Co. (St. Louis, MO), respectively, and stock solutions were made freshly for each experiment in sterile dH₂O. For drug treatment with bleomycin or doxorubicin, cells were exposed for 24 h to a concentration of each drug that produced <15% cell killing over a 72-h time period. Paclitaxel (Taxol) was prepared as a 1 mM stock in absolute ethanol.

![Fig. 1. Effect of UV irradiation on induction of wild-type p53 and MAP4 protein expression. Exponentially growing C127 cells were treated with 10 J/m² of UV irradiation. Total protein was collected and analyzed 24 and 48 h following irradiation. Loading was normalized to protein concentration, and equal transfer was measured by Coomassie Blue staining of the transferred gels. p53 and MAP4 expression was assayed by Western analysis as described in “Materials and Methods” using monoclonal antibodies Pab240 and IF5 MAP4, respectively. The Western blot shown is a representative of three separate experiments.](image1)

![Fig. 2. Effect of UV irradiation on the sensitivity to microtubule-active drugs in C127 breast carcinoma cells. Exponentially growing C127 cells were treated with 10 J/m² of UV irradiation. Twenty-four (A and B) and 72 (C and D) h following irradiation, cells were exposed to various concentrations of paclitaxel and vinblastine for an additional 72 h. Cell viability was measured with the MTT assay, as described in “Materials and Methods.” Data points, means from three separate experiments; bars, SD.](image2)
Vinblastine was dissolved in sterile distilled water. Stock solutions of all drugs were freshly prepared for each experiment and diluted to appropriate concentrations in vehicle immediately before adding to the cells.

All chemicals were purchased from Sigma unless otherwise indicated.

Cell Viability. Cell viability was assessed by the MTT assay (16, 17). MTT (Sigma) was freshly prepared as a 5 mg/ml stock in PBS. The MTT-formazan product was dissolved in 100% DMSO after a 4-h incubation, as described previously (16, 17). Absorbance was measured at 550 nm using a Dynatech.

Fig. 3. Effect of UV irradiation on the sensitivity to non-microtubule-active drugs in C127 breast carcinoma cells. Exponentially growing C127 cells were treated with 10 J/m² of UV irradiation. Twenty-four h following irradiation, cells were exposed to various concentrations of 1-β-D-arabinofuranosylcytosine (A) or doxorubicin (B) for 72 h. Cell viability was measured with the MTT assay as described in “Materials and Methods.” Data points, means from three separate experiments; bars, SD.

Fig. 4. Effect of DNA-damaging agents on p53 and MAP4 expression and on the sensitivity to microtubule active drugs in cells with mutant p53. p53 An-1 cells were grown at 38.5°C and exposed to 10 J/m² of UV irradiation, doxorubicin, or bleomycin, as described in “Materials and Methods.” A, total protein was collected and analyzed 24 h following drug exposure. p53 and MAP4 expression were assayed by Western analysis as described in Fig. 1. The Western blot shown is a representative of three separate experiments. B and C, 24 h following DNA damage, cells were exposed to various concentrations of paclitaxel or vinblastine for an additional 72 h. Cell viability was measured with the MTT assay as described in “Materials and Methods.” Data points, means from three separate experiments; bars, SD.
microplate reader MR5000 (Dynatech Laboratory Inc., Chantilly, VA). Cell viability was expressed as the percentage of the absorbance of drug-treated cells, relative to that of the vehicle-treated controls. IC$_{50}$ was defined as the concentration of drug that produced a 50% decrease in cell viability relative to the vehicle-treated controls.

Exponentially growing cells were plated into 96-well tissue culture plates. Various concentrations of diluted drugs were added to quadruplicate wells (final vehicle concentration, $\leq$1%). For experiments carried out after DNA damage, cells in logarithmic phase of growth were either UV-irradiated at 10 J/m$^2$ or treated with bleomycin or doxorubicin for 24 h to allow the induction of wild-type p53 before plating into 96-well tissue culture plates (18, 19). Cells were then exposed to various concentrations of paclitaxel or vinblastine for 72 h.

**Western Blotting.** Measurement of p53 by immunoblotting was performed as described previously, using mouse monoclonal antibody PAb240 (Santa Cruz Biotechnology, Santa Cruz, CA), which is equally reactive with denatured wild-type and mutant p53 proteins of murine and human origin (20). PAb240 was diluted in Blotto (5% milk-2% BSA, PBS, and 0.05% Tween 20) to a final concentration of 5 mg/ml. A 1:10,000 dilution in PBSBT (PBS, 1% BSA, 0.05% Tween 20) of goat-antimouse IgG (Sigma) was used as the secondary antibody in a direct chemiluminescence system (Amersham, Arlington Heights, IL).

Measurement of MAP4 by immunoblotting was performed as described previously (4), using the IF5 rat-antimouse MAP4 monoclonal antibody kindly supplied by Dr. Joanna Olmsted (University of Rochester). Rabbit-antirat IgG (Sigma) was diluted 1:10,000 dilution in PBSBT and used as the secondary antibody, which was visualized by direct chemiluminescence (Amersham).

Loading of lanes was normalized to protein concentration and complete transfer was validated by staining of the posttransfer gels with Coomassie Blue. Bands were quantitated by phosphorimaging using a Bio-Rad Molecular Imager System (5).

**Binding of Fluoresceinated Drugs.** Cells were grown either on glass coverslips or culture dishes before and after DNA damage followed by incubation with either 200 nM fluorescein-conjugated paclitaxel (Bodipy-FL Taxol®; Molecular Probes Inc., Eugene, OR) or fluorescein-conjugated vinblastine (Bodipy-FL Vinblastine; Molecular Probes). After 60 min of incubation, cells were washed free of unbound drug and visualized under a fluorescent microscope. Fluorescence intensity of bound drug was quantitated by flow cytometry and profiles were generated on a Becton Dickson FACScan analyzer with laser excitation wavelength at 488 nm (5).

**Statistical Analysis.** Statistical analysis of each dose-response curve was performed by the method of Finney (21). Accordingly, mean IC$_{50}$ values ± SDs for the inhibition of proliferation by drugs under different experimental conditions were determined by linear regression analysis of the logit-transformed data. The differences between IC$_{50}$s were determined using the Student’s $t$ test (two-tailed).

**RESULTS**

**Induction of Wild-Type p53 by UV Irradiation Leads to Decreased MAP4 Expression and Altered Sensitivity to Microtubule-active Drugs.** To determine the effect of DNA damage on p53 and MAP4 expression, we exposed C127 cells to 10 J/m$^2$ of UV irradiation. As shown in Fig. 1, UV irradiation increased the expression of wild-type p53 by 5-fold and decreased the expression of MAP4 by 5–7-fold. C127 cells were 32-fold more sensitive to vinblastine and 20-fold less sensitive to paclitaxel 24 h after UV irradiation (Fig. 2, A and B). Forty-eight h postradiation, MAP4 and p53 protein expression returned to baseline (Fig. 1). After that period of time, the differences in drug sensitivity were no longer seen (Fig. 2, A and B).
To rule out an effect of bleomycin or doxorubicin on sensitivity to antimitotic drugs independent of wild-type p53, we carried out identical assays using BRK p53-An1 cells. Treatment of p53-An1 cells with bleomycin or doxorubicin grown at 38.5°C (mutant p53) had no significant effect on the sensitivity to paclitaxel (Fig. 4B) or vinblastine (Fig. 4C).

**Induction of Wild-Type p53 by UV Irradiation Leads to Altered Cellular Binding of Microtubule-active Drugs.** To determine whether the increased sensitivity to vinblastine and decreased sensitivity to paclitaxel after DNA damage was associated with changes in cellular binding of drugs, we incubated live C127 cells with fluoresceinated paclitaxel or fluoresceinated vinblastine before and after UV irradiation. Binding was visualized by immunofluorescence microscopy. Twenty-four h after UV irradiation, C127 cells bound more fluoresceinated vinblastine than did untreated cells (Fig. 8, AC and AD). In contrast, binding of fluoresceinated-paclitaxel was decreased after UV irradiation (Fig. 8, AA and AB). No significant changes in drug binding were observed with p53-An1 cells (mutant p53) before or after UV irradiation (Fig. 8, Aa–Ad). Quantitative analysis of

C and D). UV irradiation had no effect on the sensitivity to 1-β-D-arabinofuranosylcytosine and doxorubicin, drugs that do not affect microtubule function (Fig. 3, A and B).

To exclude the effect of UV irradiation alone on drug sensitivity, we performed identical assays using BRK p53-An1 cells, which express a temperature-sensitive p53 mutant protein that is transcriptionally silent at 38.5°C (8). As shown in Fig. 4A, DNA damage by UV irradiation, doxorubicin, or bleomycin did not alter the expression of p53 and MAP4 in cells with mutant p53. DNA-damaging agents, including UV irradiation, doxorubicin, and bleomycin, did not affect the sensitivity to paclitaxel (P > 0.1; Fig. 4B). In addition, doxorubicin and bleomycin did not affect the sensitivity of p53 An-1 cells to vinblastine (P > 0.1; Fig. 4C). However, p53-An1 cells were more sensitive to vinblastine 24 h after UV irradiation (P = 0.004; Fig. 4C).

To determine whether the effect of UV irradiation on sensitivity to antimitotic drugs was a more general phenomenon, we studied several human cancer cells containing wild-type p53. Fig. 5 demonstrates that UV irradiation increased the sensitivity to *Vinca* alkaloids and decreased the sensitivity to taxanes in PA-1 human ovarian carcinoma cells (12–15) and LnCAP human prostate carcinoma cells (9–11).

**Induction of Wild-Type p53 by DNA Damage Other Than UV Irradiation Produces Decreased Expression of MAP4 and Altered Sensitivity to Microtubule-active Drugs.** To evaluate other DNA-damaging agents, we studied the effect of bleomycin and doxorubicin on the expression of wild-type p53 and MAP4 proteins. As shown in Fig. 6, treatment of C127 cells with 200 nm bleomycin or 20 nm doxorubicin (concentrations that caused <15% cell kill, data not shown) produced a 2–5-fold increase in the expression of p53 and a 5-fold decrease in MAP4, as measured by phosphorimaging.

C127 cells were then exposed to paclitaxel or vinblastine for 72 h with and without pre-exposure to the DNA-damaging agents. Fig. 7A shows that C127 cells were >20-fold more resistant to paclitaxel (IC_{50} = 160 nm) after bleomycin or doxorubicin treatment, compared to cells not previously exposed to the DNA-damaging drugs (IC_{50} = 7 nm). In contrast, Fig. 7B demonstrates that C127 cells were 30-fold (IC_{50} = 5 nm) and 60-fold (IC_{50} = 2.5 nm) more sensitive to vinblastine after doxorubicin and bleomycin treatment, respectively, compared to cells not previously exposed to the DNA-damaging drugs (IC_{50} = 160 nm).

![Diagram](image-url)
Fig. 8. Effect of UV irradiation on cellular binding of microtubule-active drugs. A, live C127 cells (AA–AD) and BRK p53-An1 cells (Au–Ad) were UV-irradiated at 10 J/m² 24 h before incubation with fluorescein-conjugated paclitaxel (200 nM) or fluorescein-conjugated vinblastine (200 nM) for 60 min. Images were taken from a fluorescent microscope under a 40× oil-immersion objective. B, cellular binding of microtubule-active drugs to live p53-An1 cells (A and B) and C127 cells (C and D) was quantitated by fluorescent-activated cell sorting as described in “Materials and Methods.” The results are representative of two individual experiments.
changes in drug binding was performed by flow cytometry and are shown in Fig. 8B.

DISCUSSION

Sensitivity to microtubule-active drugs is influenced by various factors, including P-glycoprotein-mediated mechanisms of drug efflux (22), altered tubulin structure and function (23–26), and the ability of cells to survive lethal damage due to the expression of antipapoptotic gene products (27). Several laboratories, including our own, found that mutant p53 increased the sensitivity to paclitaxel in a tissue-specific manner (5, 28–31). Our previous studies demonstrated that one of the mechanisms by which mutant p53 increases sensitivity to paclitaxel is through derepression of MAP4 (5). Because MAP4 promotes polymerization of microtubules by binding to the COOH terminus of α- and β-tubulin (32–34), p53-regulated changes in the expression of MAP4 can influence the sensitivity to drugs with mechanisms of action that affect the state of microtubule polymerization. We went on to demonstrate that overexpression of MAP4 in mouse embryo fibroblasts fully mimicked the drug sensitivity profile observed in cells with mutant p53 (5). Accordingly, we hypothesized that repression of MAP4 through induction of wild-type p53 would provide a plausible means to manipulate sensitivity to taxanes and Vinca alkaloids in cancers with endogenous wild-type p53.

MAP4 Expression Is Repressed by Induction of Wild-Type p53 through DNA Damage. p53 is known to be induced by numerous DNA-damaging agents, including UV irradiation and cancer chemotherapeutic drugs (18, 19, 35, 36). p53 regulates G1-S cell cycle arrest and may allow time for DNA repair following genomic damage (36–39). Murphy et al. (4) recently reported that MAP4 expression was transcriptionally repressed when wild-type p53 was induced by UV irradiation. This led us to investigate the influence of induction of p53 by DNA-damaging agents on MAP4 expression, microtubule polymerization, and the sensitivity to antimicrotubule drugs.

C127 murine breast carcinoma cells are transformed by BPV-1, which does not produce gene products that bind to or promote the degradation of the p53 protein (7). As shown in Fig. 1, UV irradiation of C127 cells increases expression of wild-type p53 and decreases expression of MAP4. Because UV irradiation produces a myriad of cellular responses, we investigated whether other DNA-damaging agents would produce similar effects. Accordingly, we found that bleomycin and doxorubicin both increase p53 expression and repress the expression of MAP4 (Fig. 6). In contrast, UV irradiation, bleomycin, and doxorubicin do not alter the expression of p53 or MAP4 in cells containing mutant p53 (Fig. 4A).

Repression of MAP4 by p53 Decreases Sensitivity to Paclitaxel and Increases Sensitivity to the Vinca Alkaloids. Vinca alkaloids have been used to augment the effects of radiation in a variety of human malignancies, including prostate cancer (40, 41). In contrast, the effect of paclitaxel on the sensitivity to radiation is not universal. In certain cell lines, paclitaxel treatment rendered cells more sensitive to radiation (42–45), whereas in other cells, paclitaxel did not have a significant effect (46–48).

Repression of MAP4 by p53 following UV irradiation renders C127 cells 32-fold more sensitive to vinblastine and 20-fold less sensitive to paclitaxel (Fig. 2, A and B). By 48 h postirradiation, MAP4 and p53 protein expression return to baseline (Fig. 1). After that period of time, the differences in drug sensitivity are no longer seen (Fig. 2, C and D). We also found increased sensitivity to vinblastine and decreased sensitivity to paclitaxel after UV irradiation in several human carcinoma cell lines that harbor wild-type p53, including PA-1 ovarian carcinoma cells and LnCAP prostate carcinoma cells (Fig. 5). These results suggest that this mechanism of altering drug sensitivity may be applicable to several types of human cancers.

The UV-induced decrease in sensitivity to paclitaxel appears to be dependent on a transcriptionally competent p53 because no significant changes in sensitivity were observed in BRK p53-An1 cells before or after UV exposure (Fig. 4B). In contrast, the UV-induced increase in sensitivity to vinblastine in p53-An1 cells suggests that both p53-dependent and -independent mechanisms exist (Fig. 4C).

Doxorubicin and bleomycin have similar effects in C127 cells; they increase the sensitivity to vinblastine and decrease the sensitivity to paclitaxel (Fig. 7). The effects of these drugs are not observed in p53-An1 cells, suggesting dependence on transcriptionally competent p53 (Fig. 4, A, B, and C).

MAP4 Expression May Explain Changes in Sensitivity to Microtubule-active Drugs following DNA Damage. Taxanes bind to and stabilize polymerized microtubules, leading to altered microtubular function. MAP4 promotes polymerization of microtubules by binding to the negatively charged COOH termini of α- and β-tubulin (49). We previously found that overexpression of MAP4 in mouse embryo fibroblasts increased polymerization of microtubules and cellular binding of fluorescently labeled paclitaxel (5).

Vinca alkaloids bind to tubulin monomers and inhibit microtubule polymerization (50). Repression of MAP4 by p53 promotes a shift in tubulin dynamics toward depolymerization (5). In parental mouse embryo fibroblasts with lower levels of MAP4, there were more depolymerized paracrystalline tubulin structures after treatment with Vinca alkaloids (5). Twenty-four h after UV treatment, when MAP4 expression is suppressed, C127 cells bound more vinblastine (Fig. 8, AC and AD) and less paclitaxel (Fig. 8, AA and AB) than untreated cells. There was no significant change in fluorescence intensity of bound drugs in p53-An1 cells before and after UV irradiation in the presence of transcriptionally inactive p53 (Fig. 8, AA–AD).

In summary, our studies demonstrate that induction of wild-type p53 by DNA damage can alter the sensitivity to antimicrotubule drugs and suggest that this is mediated through p53-induced repression of MAP4. These changes in drug sensitivity are consistent with the effects of MAP4 on microtubule polymerization. Because the expression of wild-type p53 and MAP4 can be regulated by treatment with DNA-damaging agents, these studies provide a rationale for the future design of combination anticancer therapies.

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REFERENCES


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