THE INDIVIDUALIZATION OF CANCER THERAPY: THE UNEXPECTED ROLE OF P53

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ABSTRACT

Our laboratory discovered that p53 can regulate the sensitivity to cancer therapies by affecting three critical aspects of cancer pharmacology: 1). The expression of drug targets; 2). the access of drugs to intracellular targets; and the response to DNA damage. We review the effects of p53 on antimicrotubule drugs through transcriptional regulation of MAP4 and stathmin (Oncoprotein 18). These two p53-regulated proteins control microtubule dynamics, regulate the sensitivity to taxanes and vinca alkaloids by changing the polymerization dynamics of tubulin and affecting the binding of drugs to microtubules. We found that overexpression of MAP4 increased microtubule polymerization and increased taxane binding and sensitivity. Overexpression of stathmin, a microtubule destabilizer, virtually abolished cellular taxane binding and increased resistance by over 1000-fold. Yet, despite an increased binding of vinca alkaloids to stathmin transfectants, we did not observe increased drug sensitivity. This was explained, at least in part, by a delay in G2/M transit. We also discovered that p53 could regulate the expression of multidrug resistance protein-1 (MRP1), a member of the ABC family of transporters that mediates the sensitivity to vinca alkaloids and anthracyclines. We found that as prostate cancer progressed from low stage/low grade to high stage/high grade there was an increased expression of both MRP1 and staining for p53, a surrogate for p53 mutations. We went on to show that p53 regulated the expression of MRP1 and that this produced resistance to doxorubicin and vinblastine. We further demonstrated that MRP1 overexpression blocked the accumulation of flutamide and hydroxy-flutamide (the active metabolite) without affecting transport of dihydrotesterone, thereby blocking access of the anti-androgen but not the androgen to intracellular androgen receptors. Finally, we reviewed the effects of DNA damage on p53 expression and MAP4 repression as a means to increase the effectiveness of breast cancer treatment. These data demonstrated the possibility of individualizing treatment based on p53 status.

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Introduction

The choice of cancer chemotherapy is often empiric, based more on the histology of the tumor than on an understanding of the molecular determinants of drug sensitivity. As a result, many patients are treated with toxic medications that fail to produce meaningful effects in terms of amelioration of symptoms or prolongation of life. Recently, important oncogenic proteins have been discovered that affect the action of cancer chemotherapeutic agents (1). These appear to be selected for the survival advantage they impart to tumors. We now appreciate that the function of several oncogenes and tumor-suppressor genes is to protect cellular viability, and that these viability factors may also lead to resistance to anticancer drugs.

p53 is one of the most commonly mutated genes in human cancer (2); loss of wild-type function through mutation or deletion can have significant effects on the way cells respond to injury, including radiation and chemotherapy (3). Levine and colleagues identified p53 as a protein involved in cellular transformation mediated by the SV40 virus (4,5). Located on chromosome 17p, alterations at the p53 locus are common in human cancer (6). For example, 75–80% of colon cancers show mutations or deletions of both p53 alleles (6). Transcriptional activation, the best-characterized function of p53, is responsible for the expression of target genes including *mdm2* (7), *GADD45* (8) and $p21^{/Waf1/Cip1}$ (9) and for many of the downstream effects of p53 activation. We found that mutations in p53 simultaneously increased the sensitivity to taxanes and decreased the sensitivity to vinca alkaloids, two classes of antimicrotubule drugs that are amongst the most frequently used anticancer agents (10). Furthermore, we demonstrated that changes in expression of genes that are transcriptionally regulated by p53 (e.g., *MAP4*[1], *stathmin*, *MRP1*) provide credible mechanisms to account for these seemingly contradictory changes in drug sensitivity (11).

Taxanes, such as paclitaxel and docetaxel, and vinca alkaloids, such as vinorelbine and vinblastine, are some of the most active drugs in the treatment of cancer, yet, only 30–50% of previously untreated patients respond to these individual agents (12). Nonetheless, clinicians have few ways of predicting who will respond and who will not. Our work suggests that the function of p53 may regulate the pharmacology of antimicrotubule drugs and thereby help predict response to chemotherapy.

p53 has also been implicated in the response to genomic and nongenomic cellular damage through its role in both growth arrest and apoptosis (3). Cells with wild-type p53 have a propensity towards

cell-cycle arrest following DNA damage, whereas cells with mutant p53 appear to bypass the G1/S check point (8). The former response is believed to allow time for repair of cellular damage, whereas the latter permits DNA replication at a time when the fate of cells is uncertain. Under circumstances associated with either overwhelming cellular damage or alarming cellular signals, wild-type p53 can activate programmed cell death. In contrast, cells with mutant p53 appear less likely to undergo apoptosis at equivalent levels of cellular damage (3). The predilection of cells to undergo apoptosis may correlate with the sensitivity to chemotherapy (13).

p53 can both activate and repress transcription; the former through binding of the wild-type protein to p53 response elements within promoter regions, and the latter by forming co-repressor complexes with mSin3A and histone deacetylase.

We studied the influence of p53 on the response to chemotherapy. Our work uncovered previously unanticipated factors that impact the efficacy of antimicrotubule drugs, leading to clinical studies designed to test the overall hypothesis that p53 status could be used to predict the response to cancer chemotherapy. These results revealed that p53 affects the access of drugs to intracellular targets, drug:target interactions, and the downstream response to cellular injury.

Results and Discussion

p53 ALTERS DRUG:TARGET INTERACTIONS. We began by studying the influence of p53 on the sensitivity to chemotherapeutic drugs. Using baby rat kidney (BRK) cells transformed with the E1A oncoprotein then transfected with a dominant-negative, temperature-sensitive p53 mutant, we found that most drugs were less effective when p53 was mutant, with the striking exception of paclitaxel (10). As shown in Figure 1, paclitaxel had greater activity at the restrictive temperature (p53 mutant). A series of control experiments confirmed that this was not due to the effect of temperature alone (Figure 1C, 1D), nor was it restricted to the BRK model. The striking finding was that the results with taxanes were exactly opposite to those obtained with the vinca alkaloids, i.e., vinca alkaloids were less active in the presence of p53 mutations.

We next investigated the mechanism(s) by which p53 changed the sensitivity to antimicrotubule drugs. Since p53 is a transcription factor, changes in expression of p53-dependent gene products might affect drug sensitivity. Whereas transcription of several genes is activated by wild-type p53, only recently have other genes been rigorously shown to

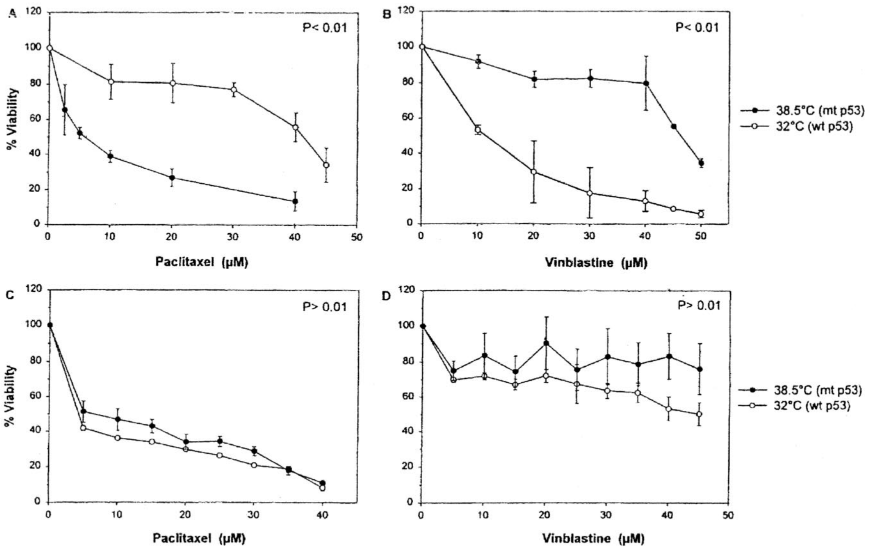
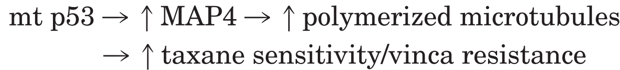


FIG. 1. p53 mutation tsp (Val[135]) increases the sensitivity to paclitaxel and decreases the sensitivity to vinblastine in BRK p53-An1 (A, B) and p53-DD[16] (C, D) cells. Cells were plated into 96-well tissue culture plates at a density of 2.4×10^4 cells per well, and grown at the restrictive (38.5°C) or shifted to the permissive temperature (32.5°C) for 24 h to allow the expression of wild-type p53. Cells were exposed to 0–50 μM drug for 48 hours and viability was measured by MTT assay. Values shown are the means $\pm$ s.e.m. of quadruplicate samples from three separate experiments.

be repressed by wild-type p53 under physiological conditions (14). As one might expect, many of these genes are directly involved in cell division and viability.

Cellular physiology is dependent on the proper assembly and organization of microtubules. Antimicrotubule drugs such as vinca alkaloids inhibit the polymerization of microtubules, whereas taxanes and related compounds inhibit microtubule depolymerization. MAPs bind to the microtubule lattice, regulate the dynamics of microtubule assembly, and the interaction of microtubules with other cellular proteins and organelles. The major MAP in non-neuronal tissues is MAP4. A single gene encodes MAP4, and multiple mRNAs are expressed in a tissue specific manner (15,16). Sequencing of the human cDNA revealed four 18-mer repeats characteristic of the microtubule-binding domains of MAP2 and tau, the major MAPs in neurons. MAP4 appears to play a critical role in defining the polymerization state of microtubules by catalyzing the polymerization of microtubules and stabilizing the polymerized form by binding to the negatively charged C-terminus of α- and β-tubulin (17).

Using a cell line expressing a temperature-sensitive p53 mutant protein (Val[5]), Murphy et al. demonstrated transcriptional repression of the *MAP4* promoter by wild-type p53 and derepression when the p53 protein was functionally mutant (14). p53 null cell lines were also shown to have high levels of MAP4, ruling out the possibility that the gene was activated by a gain-of-function mutation. Therefore, inactivation of p53 would lead to overexpression of MAP4 and favor an increase in polymerized microtubules. We reasoned that this in turn might influence the sensitivity to drugs whose mechanism of action affects the polymerization dynamics of these critical cellular components. We then tested the model that p53 mutation increased the sensitivity to taxanes and decreased the sensitivity to vinca alkaloids by releasing MAP4 from transcriptional repression, thereby favoring microtubule polymerization:



Our experiments demonstrated that p53 mutation increased the expression of MAP4 in the BRK model cell line and several others (10). In collaboration with Drs. Arnold Levine and Maureen Murphy, we then asked whether overexpression of MAP4 (against a background of wild-type p53) could recapitulate the mutant p53 phenotype with respect to sensitivity to antimicrotubule drugs. As shown in Figure 2, overexpression of p53 increased microtubule polymerization (Figure 2A), increased binding of paclitaxel (Figure 2B), and increased taxane-induced apoptosis (Figure 2C). Figure 2D demonstrates that these transfectants were more sensitive to paclitaxel and less sensitive to vinblastine than empty-vector controls. These data suggested for the first time that a tumor-suppressor protein could regulate the target for antimicrotubule drugs and thereby affect drug sensitivity.

The role of MAP4 in the sensitivity to antimicrotubule drugs was unexpected since investigators had previously focused directly on tubulin isotypes and putative drug-binding domains on β-tubulin, rather than on regulatory proteins. In fact, several lines of evidence supporting this possibility predated our work. For example, Piepmeier et al. demonstrated that injection of MAP4 or its c-terminal domain into intact cells increased microtubule bundling and produced resistance to nocadazole, a microtubule-depolymerizing drug (18). Contradictory results had been obtained earlier in a taxane-dependent, Chinese hamster ovary cell line (19), which may now be explained by recent studies demonstrating MAP4 inactivation by phosphorylation in cell lines dependent on taxanes for viability (20).

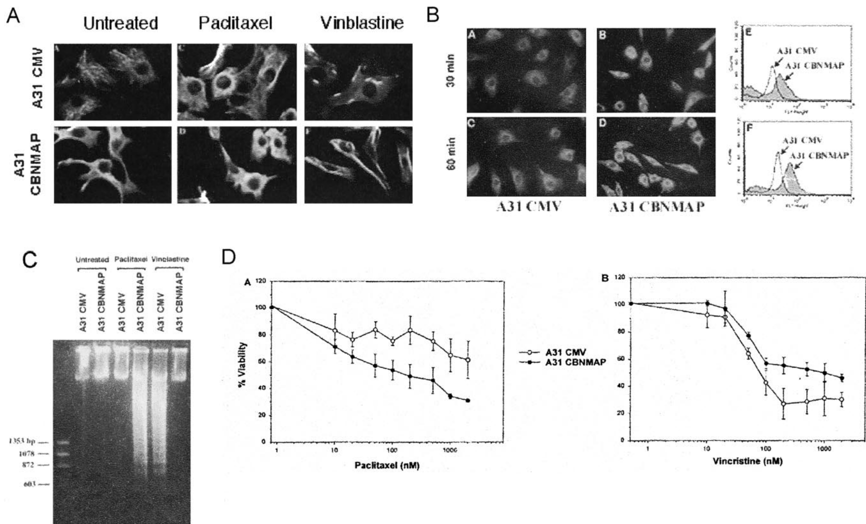
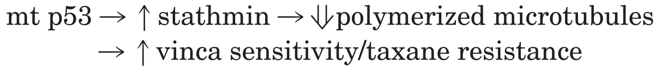


FIG. 2. MAP4 affects drug:target interactions and sensitivity to antimicrotubule agents. MAP4 transfection of murine fibroblasts (CBNMAP) increases the baseline and paclitaxel-induced polymerization of microtubules (A); increases the binding of fluorescein-labeled paclitaxel (B); increases paclitaxel- and decreases vinblastine-induced apoptosis (C) and; increases sensitivity to paclitaxel and decreases sensitivity to vincristine (D) (from Zhang et al., 1998). (A) A31 CMV (top panel) or A31 CBNMAP (bottom panel) cells were treated with vehicle, 500 nM paclitaxel, or 500 nM vinblastine for 1 h, fixed in methanol and stained with mouse anti-a-tubulin antibody DM 1A and fluorescein-conjugated goat-anti-mouse IgG. Cells were visualized by a fluorescent microscope under a 100 × oil-immersion objective. (B) A31 cells were incubated with fluorescein-conjugated paclitaxel (200 nM). At 30 min (top) and 60 min (bottom) time intervals, fluorescence images were taken from a fluorescent microscope under a 40 × oil-immersion objective. Fluorescent-activated cell sorting was performed on a Becton-Dickson FACScan analyzer with laser excitation wavelength at 488 nm. (C) DNA was extracted from A31 cells treated with vehicle, 200 nM paclitaxel or vinblastine. The DNA samples were then electrophoresed in a 1.6% agarose gel and visualized by ethidium bromide staining. (D) A31 CMV or A31 CBNMAP cells were treated with different concentrations of drugs for 72 h, and cell viability was measured by MTT assay. Values shown are the mean ± SD of quadruplicate samples and are representative of three separate experiments.

We next focused on the effect of p53 on the expression of stathmin. Stathmin (*a.k.a* Oncoprotein 18, Op18) is an 18 kDa cytosolic phosphoprotein that depolymerizes microtubules. Based on *in vitro* assays of microtubule assembly, conflicting destabilization models of either tubulin sequestration or promotion of microtubule catastrophe have been proposed, and both have been supported (21,22). Stathmin is regulated during the cell cycle by transcriptional and post-transcriptional mechanisms. To enter mitosis, stathmin must be inactivated by phosphorylation (23–27), thus promoting polymerization and aiding in the as-

sembly of the mitotic spindle (28). Like MAP4, the *stathmin* gene is repressed by wild-type p53 (29). Because of the effects of stathmin on tubulin dynamics, its transcriptional regulation by p53, and reports that stathmin is overexpressed in several types of cancer (30), we tested the model shown below (see Alli et al, 2002):



$$\text{mt p53} \rightarrow \uparrow \text{stathmin} \rightarrow \Downarrow \text{polymerized microtubules} \rightarrow \uparrow \text{vinca sensitivity/taxane resistance}$$

Overexpression of stathmin by p53 mutation or stathmin transfection decreased microtubule polymerization (Figure 3), decreased binding of taxanes (Figure 4), and produced 1000-fold resistance to paclitaxel (11). In contrast, overexpression of stathmin led to increased binding of vinblastine (Figure 5), but despite this increased drug:target

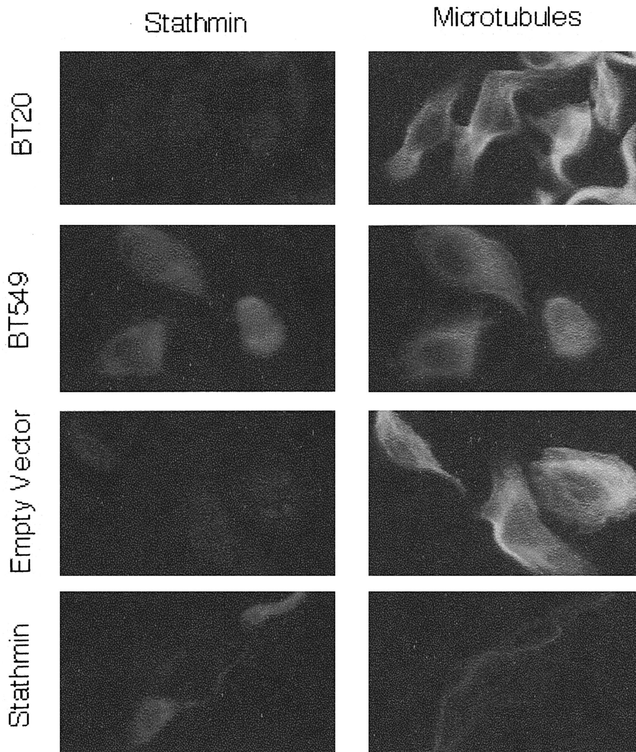


FIG. 3. Stathmin expression decreases microtubule depolymerization. BT20, BT549, empty vector-transfected BT20 and stathmin-transfected BT20 cells were permeabilized and fixed with methanol, stained for stathmin and a-tubulin using CY3- and FITC-conjugated secondary antibodies, respectively, and visualized with a fluorescent microscope under 100× oil immersion objective.

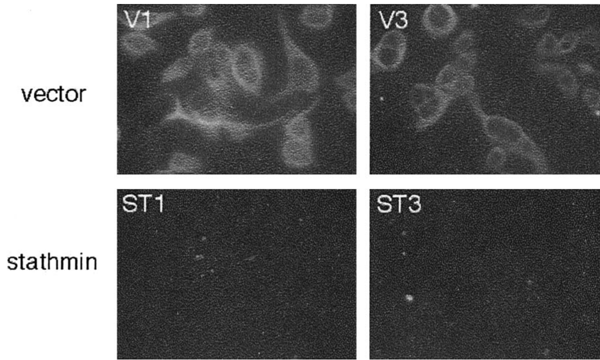


FIG. 4. Paclitaxel binding is decreased in cells overexpressing stathmin. BT20V1, BT20V3, BT20ST1, and BT20ST3 cells plated in tissue culture glass slide chambers were allowed to attach for 24 h and then treated for one hour with 20 μM fluorescein-conjugated paclitaxel. Live cells were viewed with a fluorescent microscope under 100× oil immersion objective.

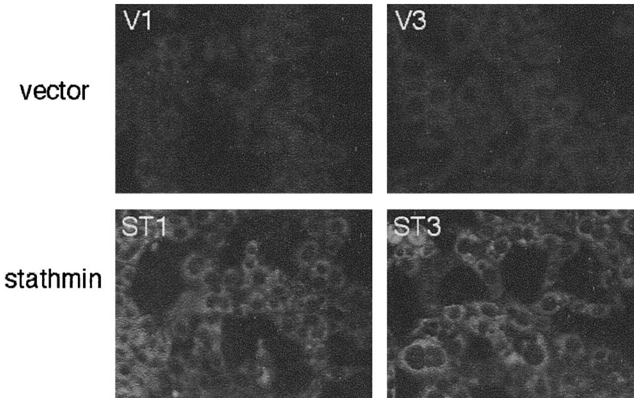


FIG. 5. Vinblastine binding is increased in cells overexpressing stathmin. BT20V1 (V1), BT20V3 (V3), BT20ST1 (ST1), and BT20ST3 (ST3) cells plated in tissue culture glass slide chambers were allowed to attach for 24 h and then treated for one hour with 20 μM fluorescein-conjugated vinblastine. Live cells were viewed with a fluorescent microscope under 40× magnification.

interaction predicted by our model, the stathmin transfectants were 5-fold less sensitive to vinca alkaloids than empty-vector controls (11).

We next attempted to understand how stathmin overexpression produces resistance to vinca alkaloids in the face of increased drug binding. Our initial results provide insights into how this might occur. For example, we measured the effect of stathmin overexpression on cell-cycle distribution and found a block at G2 (11) and a delayed transition into mitosis (Figure 6). We then compared the movement of

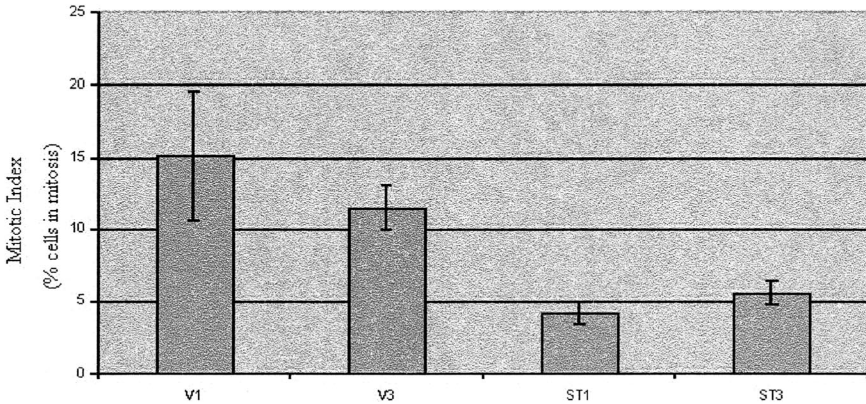


FIG. 6. Mitotic index is decreased in cell overexpressing stathmin. BT20 stathmin transfectants (ST1, ST2) or empty vector controls (V1, V2) in exponential growth were collected and centrifuged onto glass slides, then fixed and stained with 10 μg/ml 4′,6-diamidino-2-phenylindole in PBS containing 5 μg/ml RNase A. For each sample, ~200 cells were randomly counted by fluorescence microscopy and scored for mitotic cells. Percentages are expressed as mean ± SE of four individual experiments.

cells into M phase following treatment with vinblastine in vector- and stathmin-transfected cells. Stathmin transfectants treated with vinblastine showed a two- to three-fold decrease in recognition by the MPM-2 antibody, which recognizes protein epitopes specifically phosphorylated in early mitosis (Figure 7) (31). These alterations in the stathmin transfectants were not explained by a cellular attempt to tolerate overexpression of the protein, since the transfectants had a similar morphology, cell cycle transit time, cell size, shape, and viability.

Therefore, it appears that overexpression of stathmin can affect the activity of antimicrotubule drugs in two ways; 1) through altering drug binding and 2) by impeding the entry of cells from G2 into mitosis. Since stathmin must be inactivated by phosphorylation for cells to enter mitosis, we postulated that forced overexpression allowed stathmin to remain active, thereby preventing entry into mitosis where cell killing occurs. This ability to produce resistance to vinca alkaloids in the face of increased drug:target interactions suggests that the regulation of stathmin expression and function may account for a previously unanticipated mechanism of drug resistance. Furthermore, loss of normal coordination between stathmin and MAP4 expression and activities is likely to be disrupted during oncogenesis and may play an important but previously uninvestigated role in the G2/M checkpoint. Our data suggest that understanding the roles of these p53-regulated proteins will be critical to determining the ultimate prediction of sensitivity to antimicrotubule drugs.

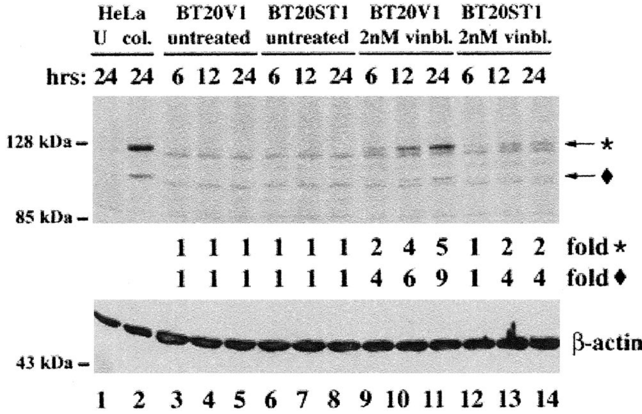
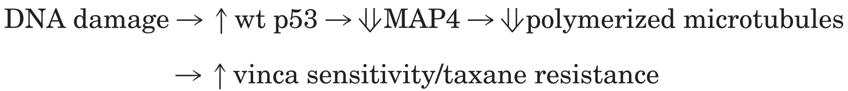


FIG. 7. Decreased expression of proteins detected by MPM-2 in stathmin-overexpressing cells treated with vinblastine. Whole cell extracts (80 μg) of BT20 stathmin transfectants (ST1, ST2) or empty vector controls (V1) either untreated (lanes 3–8) or treated with 2 nM vinblastine (lanes 9–14) were examined for proteins recognized by the MPM-2 antibody by Western blot. HeLa cells either untreated (lane 1) or treated with 50 ng/ml of colcemid (lane 2) were used as negative and positive controls, respectively. b-actin was used as a loading control. Bands were quantified by densitometry using Molecular Analyst software (BioRad).

p53 CAN ALTER THE DOWNSTREAM EFFECTS OF DRUG TARGET INTERACTIONS. Many cancer therapies act by producing DNA damage, which in turn activates p53-mediated signaling pathways. Murphy's group demonstrated that MAP4 was regulated by activation of wild-type p53 following UV irradiation. We investigated the effects of DNA damaging agents (radiation and chemotherapy) on the expression of p53, MAP4, and the effects on the sensitivity to taxanes and vinca alkaloids (32).



Our studies demonstrated that DNA damage activated wild-type p53, repressed MAP4 (Figure 8), and produced sensitivity to vinca alkaloids and resistance to taxanes (Figure 9) with minimal effects on the sensitivity to drugs that did not affect microtubules (32). In contrast, in the presence of mutant p53, DNA damage had no effect on the expression of MAP4 or on the sensitivity to antimitotic drugs (32).

These results, confirmed in several human cancer cell lines (32), led to a Phase I/II clinical trial to determine if DNA damage had similar effects in patients (33). Within 24 hours of a single dose of doxorubicin,

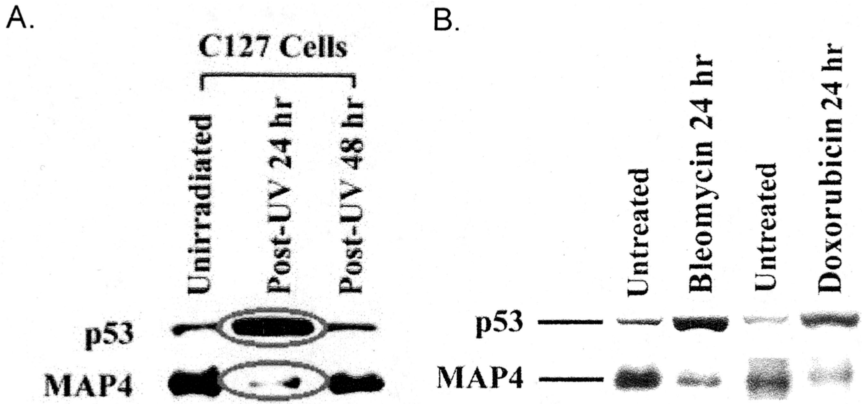


FIG. 8. Induction of wild-type p53 by UV-irradiation (A) or bleomycin and doxorubicin (B) decreases MAP4. Exponentially growing C127 cells were treated with 10 J/m^2 of UV-irradiation, or with bleomycin (200 nM) or doxorubicin (20 nM) for 24 h. Total protein was collected and analyzed after 24 hr and 48 hr following irradiation. p53 expression was assayed using monoclonal antibody Pab240 and MAP4 expression was assayed using the IF5 MAP4 monoclonal antibody by Western Blot.

p53 increased and MAP4 was repressed in 12 of 14 patients' peripheral blood mononuclear cells, consistent with the presence of wild-type p53 in normal cells. p53 also increased in four of ten breast cancer biopsies 24–48 hours following doxorubicin administration, consistent with the observation that between 40–50% of breast cancers harbor p53 mutations (34,35). Although the number of patients was too small to draw firm conclusions, those patients whose tumors demonstrated MAP4 repression appeared to have the greatest likelihood of response to therapy.

p53 REGULATES ACCESS OF DRUGS TO INTRACELLULAR TARGETS. We found that p53 could decrease access of drugs to their intracellular targets through regulating the expression of MRP1 (*ABCC1*) (36). While investigating the expression of drug-resistance gene products in 95 human prostate cancer specimens, we found a progressive increase in expression of p53, MRP1 and topoisomerase II-α as a function of advancing stage and grade of disease (37). These data were recently confirmed by Van Brussel et al. in organ-confined, locally advanced and disseminated prostate cancer (38). We hypothesized that p53 might regulate the expression of the *MRP1* gene, thereby accounting for these clinical findings. To test this hypothesis, we transfected LNCaP human prostate cancer cells with a dominant-negative, temperature-sensitive p53 mutant (valine^{138}), isolated transfected clones (LVCaP) and measured the effect on *MRP1* expression.

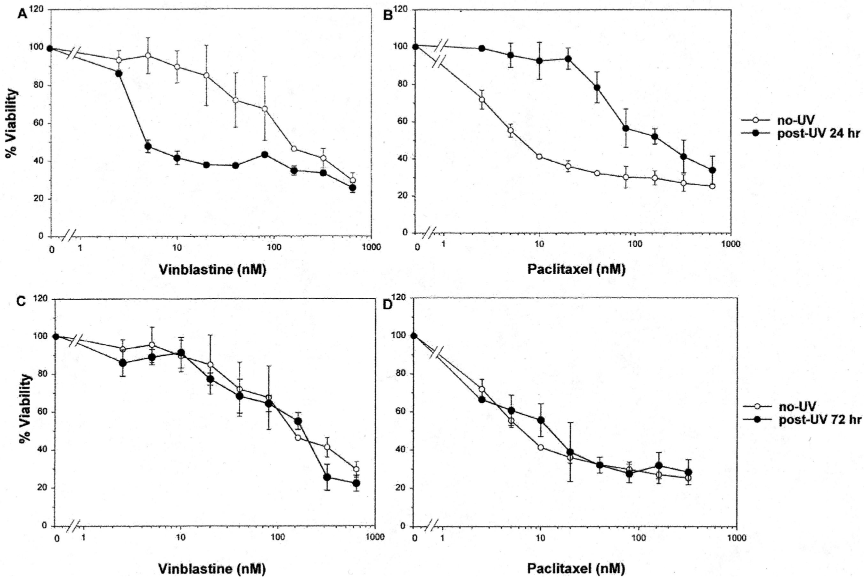


FIG. 9. UV-irradiation increases the sensitivity to vinblastine and decreases the sensitivity to paclitaxel. Exponentially growing C127 cells were treated with 10 J/m^2 of UV-irradiation. After 24 h (A and B) and 72 hr (C and D) following irradiation, cells were exposed to various concentrations of paclitaxel and vinblastine for 72 hr. Cell viability was measured with the MTT assay. Each point represents the means ± S.D. of quadruplicate samples and are representative of three separate experiments.

These results demonstrated that wild-type p53 repressed endogenous *MRP1* gene expression at the level of mRNA and protein, and that the expression was lost with p53 inactivation (Figures 10 and 11). We found similar results in other cell lines where p53 was inactivated by the

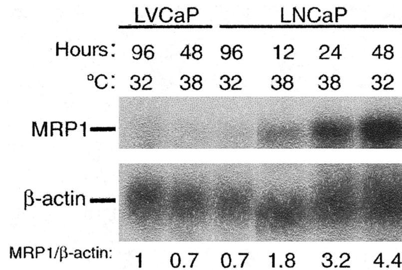


FIG. 10. p53 regulates *MRP1* mRNA expression. LVCaP cells were cultured at 38.5°C, shifted to 32°C for 24 hours, then shifted to 38.5°C for 12, 24, and 48 hours. Twenty μg of total RNA from each sample were electrophoresed, blotted onto nitrocellulose, and probed for MRP. An a-^{32}P-labeled b-actin was used to determine RNA loading.

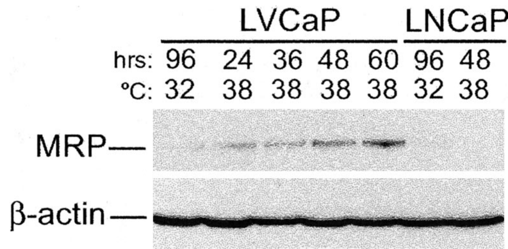


FIG. 11. p53 regulates the expression of MRP protein. Identical amounts (100 μg) of total protein were resolved using a 6% SDS-polyacrylamide gel, transferred onto a polyvinylidene difluoride membrane, and probed with monoclonal anti-human MRP and monoclonal anti-human b-actin antibodies.

human papilloma virus E6 protein or by a dominant-negative p53 mutation (36). Wang and Beck had contemporaneously found that wild-type 53 repressed *MRP1* promoter activity and mRNA expression (39).

We also recognized that certain structural features of antiandrogen medications resembled substrates of MRP1. Since p53 is altered at the time of diagnosis in 20–50% of prostate cancer patients, we studied whether or not MRP1 could affect the transport of flutamide, a prototype antiandrogen used to treat hormone-sensitive prostate cancer (40). These studies demonstrated that flutamide and the active metabolite, hydroxyflutamide, were rapidly transported by MRP1. In contrast, dihydrotestosterone transport was not affected by p53 mutation and MRP1 expression (40). Thus, in the presence of p53 mutation, the up-regulation of MRP1 in previously untreated human prostate cancer could produce resistance to both hormonal treatment and chemotherapy.

The significance of our findings was highlighted by experiments demonstrating that p53 mutation decreased accumulation of the natural MRP1 substrate, leukotriene C4 (Figure 12A), as well as doxorubicin (Figure 12B), thereby producing significant drug resistance (Table 1).

SUMMARY. Our initial interests in the role of p53 in drug sensitivity led to a more detailed understanding of the mechanisms by which this tumor suppressor gene can affect the pharmacology of anticancer drugs. We next plan to build on this work to determine how to more accurately predict responsiveness to vinca alkaloids and taxanes in the clinic. Therefore, through recognition of the role of p53-induced molecular changes in drug sensitivity, it may be possible to relate genetic events in human tumors to the choice of appropriate therapies, thereby increasing the likelihood of achieving meaningful results compared to those obtained with empirically-derived treatments.

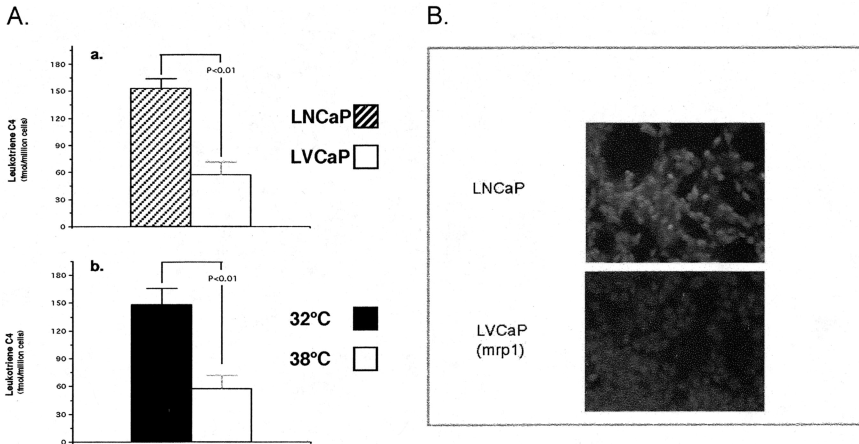


FIG. 12. Leukotriene-C4 and Doxorubicin accumulation is decreased in p53-mutant LVCaP cells. A. Leukotriene-C4. Cells were grown at 37°C (LNCaP) or 32°C (LVCaP). 80% confluent LVCaP cells were shifted to 38°C (mt p53) and incubated for 60 hours. Cells were incubated with [^{3}H]-leukotriene-C4 for 2 hr at 38°C, then cooled on ice, washed three times with ice-cold PBS, and dissolved in 0.25 ml 1% SDS. Radioactivity was determined by scintillation counting. *a.* leukotriene-C4 accumulation in LVCaP and LNCaP cells grown at 38°C. *b.* leukotriene-C4 accumulation in LVCaP cells grown at 32°C (wt p53) and the same cells grown at 38°C (mt p53). B. Doxorubicin Cells seeded in 24-well plates were grown at 37°C (LNCaP) or 32°C (LVCaP). When cells were 80% confluent, LVCaP cells were shifted to 38°C (mutant p53) and incubated for 60 hours. Cells were then incubated with 2 μM doxorubicin for 2 hr at 37°C (LNCaP) or 38°C (LVCaP). At the end of incubation, cells were washed three times with PBS, then observed under a fluorescence microscope with 100× magnification.

TABLE 1
Effect of p53 Status on Drug Sensitivity

Drug	Cell Line		*p* value
	LVCaP*	LNCaP†	
	IC_{50} (*nM*)		
Vincristine	240 ± 43	8.3 ± 0.9	0.001
Doxorubicin	360 ± 28	180 ± 1.0	0.01

* LNCaP cells transfected with a dominant-negative, temperature-sensitive p53 mutant.
† Human prostate cancer cells.

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DISCUSSION

Howley, Boston: In the triple negatives, do you have a sense of what percentage are p53-negative and what percentage are p53-positive?

Hait, New Brunswick: In our study so far, they've come out pretty much consistent with the report in the literature that about 60% of the tumors maintain wild-type p53 function. And the pathway seems to be intact, because in the data that I couldn't show, when you give the DNA damaging drug you activate p53 and you repress MAP 4.

Schiffman, Providence: Bill that was a wonderful talk. Any differences in the neurotoxicity of the vinca alkaloids or taxones in the face of the different p53 mutations?

Hait: So, that's a great question, Fred. So, we have not seen more neurotoxicity because you might be afraid if you change the binding to the target you would have more severe side effects. We've not seen it. In the first study the only complication that we saw was, perhaps, more pulmonary emboli. We saw maybe two, and we thought that was somewhat unexpected, but otherwise we didn't see more neutropenia, more neurological side-effects. Somewhat surprising, but we didn't see it.

Boyer, New Haven: Bill, I enjoyed your talk. Can you tell us, is the effect of p53 specific for Mrp1? Of course there are other Mrp's in these cells. There's also p-glycoprotein (MDR1).

Hait: Way back, it was shown, but it's been difficult to confirm that wild-type p53 could also repress MDR1 or p-glycoprotein. And we found that it's cell-context specific. Sometimes it's because the mutant p53 can be an activating mutation, it gets very complicated. In terms of the other mrp family members, I don't believe it's been studied.