Characterization of the p53 Tumor Suppressor Pathway in Cell Lines of the National Cancer Institute Anticancer Drug Screen and Correlations with the Growth-Inhibitory Potency of 123 Anticancer Agents


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ABSTRACT

In the present study, we report the characterization of the p53 tumor suppressor pathway in the 60 cell lines of the National Cancer Institute (NCI) anticancer drug screen, as well as correlations between the integrity of this pathway and the growth-inhibitory potency of 123 anticancer agents in this screen. Assessment of p53 status in these lines was achieved through complete p53 cDNA sequencing, measurement of basal p53 protein levels and functional assessment of (a) transcriptional activity of p53 cDNA from each line in a yeast assay, (b) γ-ray-induced G1 phase cell cycle arrest, and (c) γ-ray-induced expression of CHIP/WAF1, GADD45, and MDM2 mRNA. Our investigations revealed that p53 gene mutations were common in the NCI cell screen lines: 39 of 58 cell lines analyzed contained a mutant p53 sequence. cDNA derived from almost all of the mutant p53 cell lines failed to transcriptionally activate a reporter gene in yeast, and the majority of mutant p53 lines studied expressed elevated basal levels of the mutant p53 protein. In contrast to most of the wild-type p53-containing lines, cells containing mutant p53 sequence were also deficient in γ-ray induction of CHIP/WAF1, GADD45, and MDM2 mRNA and the ability to arrest in G1 following γ-irradiation. Taken together, these assessments provided indications of the integrity of the p53 pathway in the 60 cell lines of the NCI cell screen. These individual p53 assessments were subsequently used to probe a database of growth-inhibitory potency for 123 “standard agents,” which included the majority of clinically approved anticancer agents. These 123 agents have been tested against these lines on multiple occasions, and a proposed mechanism of drug action had previously been assigned to each agent. Our analysis revealed that cells with mutant p53 sequence tended to exhibit less growth inhibition in this screen than the wild-type p53 cell lines when treated with the majority of clinically used anticancer agents: including DNA cross-linking agents, antimitabolites, and topoisomerase I and II inhibitors. Similar correlations were uncovered when we probed this database using most of the other indices of p53 status we assessed in the lines. Interestingly, a class of agents that differed in this respect was the antimitotic agents. Growth-inhibitory activity of these agents tended, in this assay, to be independent of p53 status. Our characterization of the p53 pathway in the NCI cell screen lines should prove useful to researchers investigating fundamental aspects of p53 biology and pharmacology. This information also allows for the large-scale analysis of the more than 60,000 compounds tested against these lines for novel agents that might exploit defective p53 function as a means of preferential toxicity.

INTRODUCTION

The Developmental Therapeutics Program of the NCI2 uses 60 human cell lines derived from cancers of the colon, breast, ovary, lung, kidney, central nervous system, skin, and bone marrow as a screen to discover novel anticancer agents (1–3). The chemical repository from which compounds are tested for analysis consists of approximately 300,000 agents and is growing. Such agents are submitted for testing from a wide variety of sources, including individual investigators, biotechnology, and pharmaceutical companies; the agents are not limited to synthetic agents but include natural products. Approximately 60,000 agents have been tested in the NCI anticancer drug screen, and the rate of testing averages approximately 10,000 compounds per year. Of these 60,000 agents, approximately 8,000 have been considered for analysis in additional in vitro and in vivo assay systems, including studies in a panel of human tumor xenografts established in nude mice (1–4). To date, five drugs (UCN-01, flavopiridol, the spicamycin analogue KRN 5500, a quinocarcycin analogue, and a depsipeptide) have been selected for Phase I clinical evaluation based in part on activity in the NCI anticancer drug screen.

The NCI anticancer drug screen evaluates the growth-inhibitory effects of a compound in 60 cancer cell lines. Growth inhibition is measured in a 96-well microtiter format on the basis of cellular protein content (1–3). The growth fraction relative to a vehicle control is measured 48 h after the introduction of the compound at each of five concentrations covering a 4-log dose range. The dose-response curves are used to determine the GI50. Reducing each experiment to an array of 60 GI50 results in a pattern of activity for each tested compound. These activity patterns, also called “fingerprints,” have been exploited using display techniques such as the “mean graph” (1–3); the COMPARE algorithm, which searches among the database of tested compounds for agents with similar activity patterns (5–7); and the DISCOVERY program package, which incorporates additional analytical applications (8, 9). Taken together, the COMPARE and DISCOVERY programs have proven useful in classifying unknown compounds as topoisomerase inhibitors, antimitotics, or pyrimidine biosynthesis inhibitors (5–8, 10).

The 60 cell lines in the NCI anticancer drug screen are presently undergoing extensive molecular characterization to better define them in terms of the status of tumor suppressor genes, oncogenes, enzymes, and/or biological processes commonly altered in human cancer. This molecular search aims to uncover the basis of the differential chemosensitivity responses of the lines in the screen and may ultimately shape new approaches to the discovery of “lead compounds” with preferential activity toward cancer cells with defined molecular de-

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2 The abbreviations used are: NCI, National Cancer Institute; GI50, dose of agent required to inhibit growth of the cells by 50% of the vehicle control sample following a 48-h continuous exposure.
fects. Detailed knowledge of the molecular characteristics of these cell lines might also uncover interrelationships between individual components or pathways. Published examples of recent molecular dissection efforts in the NCI cell screen lines that have in turn uncovered chemosensitivity relationships include the analysis of P-glycoprotein and, recently, the ras oncogene (11–15).

In the present study, we report the status of the p53 tumor suppressor pathway in the 60 cell lines of the NCI anticancer drug screen. Our interest in the characterization of the p53 pathway in these cell lines was stimulated by (a) the high frequency with which p53 is altered in human tumors (16–18), and (b) the fact that we and others have found that p53 dysfunction can in some instances alter sensitivity to ionizing radiation and at least some chemotherapeutic drugs (19–27). The goals of our study were to (a) provide a detailed picture of the status of the p53 pathway in these cell lines and (b) to use this knowledge to uncover the impact of p53 status on the growth-inhibitory activity of a set of 123 “standard agents” that included most of the clinical agents approved for cancer treatment. These 123 agents have been tested in this primary screen on multiple occasions and have been assigned putative mechanisms of action (5, 8, 10).

Our assessment of p53 status in the NCI screen lines revealed that p53 mutations were common and that the majority of these mutant p53 cell lines lacked p53-dependent transactivation function as well as the ability to arrest in G1 following γ-irradiation. Also, in contrast to almost all of the wild-type p53 cell lines studied, the majority of the mutant p53 lines exhibited elevated basal levels of the mutant p53 protein. By using these assessments to investigate the growth-inhibitory activity of 123 standard agents of putatively known mechanism of action (5, 8, 10), we found that the mutant p53 cell lines tended, on average, to be less sensitive than the wild-type p53 lines to the majority of alkylating agents, DNA/RNA antimetabolites, and topoisomerase I and II inhibitors tested in this assay. A class of agents that differed in this respect, however, was the set of antimitic agents, including paclitaxel and vincristine. Our results suggest that for the majority of agents within the antimitotic class, p53 status was not an indicator of growth-inhibitory activity in the NCI screening assay.

**MATERIALS AND METHODS**

**Cell Lines and in Vitro Chemosensitivity Testing.** The collection and basic characterization of the 60 cell lines in the NCI anticancer drug screen has been extensively described (1–3). All cell lines were grown in RPMI 1640 that contained 5% fetal bovine serum with 5 mM l-glutamine, and cells were routinely replaced in culture every 20 passages with samples from cryopreserved stocks. Compounds were screened for their effect on cell growth using a 48-h assay with sulforhodamine B assay essentially as described previously (1–3). The measure of chemosensitivity referred to in this report is the GI50 value. Each agent tested in 2–460 independent experiments (median, 5 experiments). All 60 cell lines were assayed in parallel for each agent evaluated, and routine quality control criteria were used to exclude unsatisfactory dose-response curves (1–3, 5).

**Statistical Analysis.** Because p53 mutation status is a binary parameter and other p53-associated characteristics could reasonably be rendered as binary, and because the GI50 measurements for each drug do not all follow a normal distribution, we chose the Wilcoxon (Kruskal-Wallis) rank sum test (normal approximation) to measure the strength of the association between these binary parameters and the GI50 activity values. High levels of an individual characteristic (e.g., high basal p53 protein levels) were coded as 1 and low values as 0, thus dividing the lines into two groups with respect to that characteristic. In the case of p53 mutation status, wild-type cell lines were coded as 1 and mutant cell lines as 0. The one heterozygote (HCT-15) was coded as 0 for consistency with the expected dominant-negative effect of the mutant p53 protein. The threshold value for each of the other characteristics measured is given in the legend of Fig. 8.

A program for doing large numbers of Wilcoxon rank sum calculations (normal approximation) was written by SAS (SAS Institute, Cary, NC) for these analyses. The Wilcoxon rank sum test provides a nonparametric test of the null hypothesis that for any given characteristic, the cells coded as 0 have the same sensitivity to an agent as do the cells coded as 1. For the Wilcoxon p value (range, 0–1), a number less than 0.5 indicates a tendency for the mutant cells to be more sensitive than the wild-type cells; a number greater than 0.5 indicates the opposite. Formally, a p value less than 0.025 or greater than 0.975 is required to reject the null hypothesis of equal median sensitivities at the 5% level of confidence (two-sided). However, because the 60 cell lines do not represent a random sample selected from an assumed underlying population, the p value as used here should be thought of as a heuristic parameter indicating tendency, rather than as a formal statistical entity.

**p53 cDNA Sequencing.** Polyadenylated mRNA was extracted from cells using a MicroFast Track mRNA isolation kit (Invitrogen) and cDNA prepared using a First-Strand cDNA synthesis kit (Pharmacia) according to the manufacturers’ recommendations. Three overlapping p53-specific PCR primers covering the entire open reading frame were used to bidirectionally sequence the p53 cDNA using Taq dyeoxy sequencing methodology on an Applied Biosystems model 373A automated sequencer (Applied Biosystems; Ref. 28).

**Functional Analysis of p53 cDNA in Yeast.** Functional analysis of separated p53 alleles in yeast (FASAY) was performed essentially as described previously (28). Briefly, the FASAY assay measures the transcriptional activity of p53 cDNA from the individual cell lines in a yeast survival assay. The assay uses three centromeric plasmids. The first plasmid, pLS76 (positive control), contains a LEU2 gene for selection of transformed yeast on plates lacking leucine, and full-length wild-type human p53 cDNA expressed under the control of the ADH1 promoter. The second plasmid, pSS16, is identical to pLS76 except that the wild-type p53 sequence from codons 68–347 is replaced by the URA3 gene, which confers growth on plates lacking uracil. The third plasmid, pSS1, contains a HIS3 gene under the control of a single p53 binding site derived from the ribosomal gene cluster (29). Input p53 was generated from polyadenylated mRNA extracted from cells using a MicroFast Track mRNA isolation kit (Invitrogen) and cDNA prepared using a First-Strand cDNA synthesis kit (Pharmacia). p53 cDNA was PCR amplified using Pfu polymerase (Stratagene), and yeast was cotransfected with the PCR-generated p53 and HindIII/Sal-restricted plasmid pSS16 (which releases the URA3 gene segment). Repair of the gapped pSS16 plasmid with the p53 derived from the input p53 PCR product occurs in vivo through homologous recombination. Transformants that have successfully repaired pSS16 are selected on media lacking leucine, and such replicates lack growth on uracil-minus media. If the yeast has also been transfected with pSS1, then growth on media lacking histidine identifies colonies that contain transcriptionally active p53. Leu+ colonies containing only wild-type p53 sequences grew successfully on plates lacking histidine. Leu+ colonies containing only p53 “loss-of-function” mutations failed to grow on plates lacking histidine. Leu+ colonies derived from p53 cDNA prepared from cancer cells containing heterozygous p53 gene status yield approximately 50% His+ colonies (28).

**Gel Electrophoresis and Western Blotting.** Protein isolation and analysis were performed essentially as described (20). Briefly, cells were lysed on ice for 30 min in 1% NP-40 prepared in PBS that contained 10 µg/ml leupeptin, 10 µg/ml aprotonin, 2 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 1 mM sodium orthovanadate, 10 mM sodium fluoride, and 5 mM sodium PPi. Protein determination was performed using the BCA protein assay kit according to the manufacturer’s instructions (Pierce). One hundred µg of total cell protein were loaded onto SDS-polyacrylamide gels, and then proteins were transferred to Immobilon membranes (Millipore) using semidyed blotting techniques. Membranes were blocked for 30 min in 5% skim milk, probed for 1 h with the mouse monoclonal p53 primary antibody, Pab 1801, which recognizes an epitope that resides between amino acids 32 and 79 of p53 (Oncogene Science), and then probed with a sheep antimouse horseradish peroxidase secondary antibody (Amersham Corp.). Antibody reaction was revealed using chemiluminescence detection according to the manufacturer’s recommendations (Amersham Corp.). Proliferating cell nuclear antigen was probed for as an additional control for gel loadings and was found not to vary by more than 25% between the different blots analyzed (data not shown). Comparisons between individual blots was accomplished by including 100 µg of total cell protein from the wild-type p53 cell line, WMN, and the mutant p53 cell line, CA46 (248 RUQ), on each blot (20, 21). The basal levels of p53 in each line were classified into two groups according to the level of p53 expression in WMN...
cells (wild-type p53); low/negligible basal p53 levels (<2-fold that seen in WMN cells) and high basal p53 levels (≥2-fold that seen in WMN cells). High basal p53 levels were similar to those seen in the mutant p53 line, CA46, which has previously been reported to express elevated levels of mutant p53 (20, 21).

**Flow Cytometry.** Twenty-four h prior to commencement of the G1 arrest assay, plated cells were trypsinized and replated at 10–20% confluence. Twenty-four h later, floating cells were decanted from the plates, and fresh medium was added. For suspension cultures, exponentially growing cells were diluted to 5 × 10^6 cells/ml. Cells were irradiated at room temperature with 6.3 or 12.6 Gy of γ-rays using a 32P Cs source (5.25 Gy/min; 1 Gy = 100 rads) and then incubated for 17 h at 37°C in the presence or absence of the microtubule inhibitor nocodazole (0.1–0.4 μM/ml; Aldrich). Medium containing floating cells was combined with cells trypsinized from the plates and then centrifuged. Cells were then washed once in ice-cold PBS and fixed in 70% ethanol for a minimum of 2 h. Cells were rehybridized by being washed in PBS and then resuspended in propidium iodide (25 μg/ml; Sigma) that contained RNase A (500 units/ml; Sigma) before incubation at 37°C for 30 min. Cell cycle analysis was performed on a Becton Dickinson FACScan flow cytometer using the SOBR program provided by the manufacturer; 3–5 S-phase peaks were used to fit the model, and at least 15,000 cells were used for each analysis (Becton Dickinson). In some cases, nocodazole was included in the assay to prevent any cells that might break through the G1 checkpoint from entering G0 of the second cell cycle. Thus, the population of cells in G1, 17 h after incubation with nocodazole reflects cells in the first G1 phase (20, 21). G1 arrest was quantitated as the percentage of the control G1 population that remained in G1, 17 h after irradiation plus incubation with nocodazole. G1 arrest responses were grouped into three classes: class 1 cells showed strong G1 arrest (>20% of the original G1 population); class 2 cells showed intermediate G1 arrest (between 10 and 20% of the original G1 population); and class 3 cells showed weak or no G1 arrest (<10% of original G1 population).

**RNA Isolation and Dot-Blot Hybridization.** The induction of WAF1/CIP1, GADD45, and MDM2 mRNA was assessed 4 h after exposure to 20 Gy of γ-rays essentially as described (30, 31). Briefly, exponentially growing and irradiated cells were lysed in situ using 4 M guanidine thiocyanate. Polyadenylated mRNA was prepared from total RNA using oligo(dt)-cellulose chromatography. Eight incremental 1:2 dilutions of the polyadenylated mRNA samples were blotted onto Nytran filters (Schleicher and Schuell) using a Hybi-dot manifold (Life Technologies, Inc., Gaithersburg, MD). Membranes were irradiated with UV light (700 J/m² at 254 mm). The first four dilutions were probed with either CIP1/WAF1 (pZL-WAF1), GADD45 (pHulB2), or MDM2 (FLA) cDNA 32P-labeled probes. The CIP1/WAF1 and MDM2 probes were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). The last four dilutions were hybridized with 35S-labeled polythymidylate probe and also a 32P-labeled probe to glyceraldehyde-3-phosphate dehydrogenase, which confirmed equivalent loadings between individual samples. The level of mRNA induction relative to unirradiated control samples for each transcript was grouped into three classes: class 1 cells showed strong mRNA induction (≥4-fold above basal levels); class 2 cells showed intermediate mRNA induction (between 2-fold and 4-fold); and class 3 cells showed weak or no mRNA induction (≤2-fold).

**RESULTS**

**p53 cDNA Sequencing.** p53 sequencing was performed on cDNA prepared from polyadenylated mRNA extracted from each cell line. Three overlapping p53-specific primer pairs were used to bidirectionally sequence the entire p53 cDNA using Taq polymerase with the aid of automated DNA sequencing procedures (see “Materials and Methods”). Of the 58 cell lines sequenced, 18 (31%) were found to contain wild-type p53, 39 (67%) contained only mutant p53 sequence, and 1 (HCT-15) was heterozygous for p53 mutation (Table 1). With the exception of the prostate lines, each tissue set contained at least one wild-type p53 cell line (Table 1). The melanoma and renal cell line panels contained a higher proportion of wild-type p53 lines [melanoma, 5 of 8 (63%); renal, 4 of 8 (50%)] compared to the remaining tissue sets (9 wild-type lines of 41 (28%); melanoma, p value = 0.033; renal, p value = 0.183; Fisher’s exact test, two-tail).

The majority of p53 mutations observed in the NCI screen lines were missense mutations [33 of 40 examples (83%); the most frequently observed missense mutations occurred at codons 248 (5 examples), 266 (4 examples), and 273 (5 examples; Fig. 1). Of the four lines with a codon 266 mutation (G/E), two were of the same origin: MDAMB435 and MDAN. These lines differ in that MDAN is a Her-2/neu-transfected derivative of MDAMB435 (32). Analysis of all p53 mutations revealed a preponderance of G/A [17 of 41 (41.5%)] and G/T [5 of 41 (12%)] transitions (Table 2). Interestingly, one cell line harbored two base substitutions within the same codon (SK-MEL28: TGT/GTT), resulting in a change in a cysteine to a valine at amino acid position 145 (Table 1). Internal sequence deletions were also common [5 of 41 (12%); whereas C/A and G/C transitions were relatively infrequent (Table 2). Also commonly observed among the 58 lines analyzed was a polymorphism at codon 72 [10 of 58 lines (24%); data not shown].

With only two exceptions, all of the p53 mutations resided within the central evolutionarily conserved sequence-specific DNA binding domain of p53 (Fig. 1; Ref. 33 and references therein). The two mutations seen outside this sequence-specific DNA binding domain were found at codon 309 (P/A transition) in the lung cancer cell line NCI-H226 and codon 336 (E to stop) in the renal cancer cell line SN12C (Table 1; Fig. 1). The codon 336 mutation would lead to a truncated p53 protein lacking most of the region required for p53 oligomerization (amino acids 319–360), as well as a putative DNA damage recognition region (amino acids 311–393; Refs. 34 and 35).

**Assay of p53 cDNA Function in Yeast.** The transcriptional activity of p53 cDNA from each line was assessed in a yeast growth assay that selects for the p53-dependent expression of a survival gene that confers growth in the absence of histidine (Ref. 28; see “Materials and Methods”). Fifty-four of the 60 cell lines in the NCI screen were analyzed in this yeast growth assay. p53 cDNA derived from 17 of these lines was capable of conferring growth in the absence of histidine. Fifteen of these lines contained wild-type p53 sequence, whereas the other 2 lines (RPMI8226 and SKMEL2) contained mutations in p53 (Table 1). p53 cDNA derived from 36 lines failed to confer growth on media lacking histidine, and all of these lines contained mutant p53. These results supported the conclusion that these mutations were all loss-of-function mutations. p53 in one of these lines, K562, was not sequenced in our studies; however, this line has been reported to contain mutant p53 by other workers (18). p53 cDNA derived from HCT15 cells yielded approximately half the number of yeast colonies compared to yeast transformed with only wild-type p53 cDNA. This latter result was consistent with the heterozygous p53 gene status of HCT15 cells (Table 1; Ref. 28). p53 cDNA from two cell lines with mutations outside of the sequence-specific DNA binding domain of p53 (SN12C, 336 E/stop, and NCIH226, 309 P/A) also failed to transcriptionally activate the p53 reporter gene in yeast.

**p53 Protein Levels and Relationship to p53 Status.** Basal levels of the p53 protein were determined from exponentially growing cells by means of Western blotting using the p53 mouse monoclonal antibody Pab1801. This antibody recognizes an epitope that resides in the central evolutionarily conserved sequence-specific DNA binding domain of p53 (Fig. 1; Ref. 28; see “Materials and Methods”). Fifty-four of the 60 cell lines in the NCI screen were analyzed in this yeast growth assay. p53 cDNA derived from 17 of these lines was capable of conferring growth in the absence of histidine. Fifteen of these lines contained wild-type p53 sequence, whereas the other 2 lines (RPMI8226 and SKMEL2) contained mutations in p53 (Table 1). p53 cDNA derived from 36 lines failed to confer growth on media lacking histidine, and all of these lines contained mutant p53. These results supported the conclusion that these mutations were all loss-of-function mutations. p53 in one of these lines, K562, was not sequenced in our studies; however, this line has been reported to contain mutant p53 by other workers (18). p53 cDNA derived from HCT15 cells yielded approximately half the number of yeast colonies compared to yeast transformed with only wild-type p53 cDNA. This latter result was consistent with the heterozygous p53 gene status of HCT15 cells (Table 1; Ref. 28). p53 cDNA from two cell lines with mutations outside of the sequence-specific DNA binding domain of p53 (SN12C, 336 E/stop, and NCIH226, 309 P/A) also failed to transcriptionally activate the p53 reporter gene in yeast.
Table 1 p53 gene status in the cell lines of the NCI anticancer drug screen

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<th>Cancer type</th>
<th>p53 sequence codon change(a)</th>
<th>p53 sequence base change</th>
<th>p53 sequence amino acid change</th>
<th>Yeast assay(b)</th>
<th>Doubling time(c)</th>
<th>% in G1, % in S, % in G2/M</th>
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\(a\) Determined through complete bidirectional p53 cDNA sequencing using three overlapping PCR primer sets as described in "Materials and Methods."

\(b\) Transcriptional activity of the DNA binding domain of the p53 cDNA in a yeast-based survival assay determined as described in "Materials and Methods."

\(c\) Doubling time was calculated from the rate of growth of cells in the sulforhodamine B microtiter plate assay (see "Materials and Methods."

\(d\) Cell cycle distribution of exponentially growing cultures defined by flow cytometry as described in "Materials and Methods."

The level of p53 expression in WMN cells (wild-type p53): class 1, high basal p53 levels (>2-fold that seen in WMN cells); and class 2, low/negligible basal p53 levels (<2-fold that seen in WMN cells (Table 3)). Twenty-nine lines (53%) exhibited high basal level expression of p53; of these, 24 lines (89%) contained only mutant p53, 3 lines were wild-type for p53 (OVCAR4, IGROV, SF539), and 1 line (HCT15) was heterozygous for p53 mutation (Fig. 2 and Table 3).
Table 2: Type and frequency of p53 mutations in the NCI cell screen lines

<table>
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<th>Alteration</th>
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<tr>
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</tr>
<tr>
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<td>Insertions</td>
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<tr>
<td>Total</td>
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Fig. 1. Spectrum and position of p53 mutations in cell lines of the NCI anticancer drug screen. Shown is a diagram of the 393 amino acid nuclear p53 phosphoprotein with functional domains highlighted. The amino-terminal transactivating domain (amino acids 20—42), the central conserved DNA binding domain (amino acids 100—293), and the carboxyl-terminal oligomerization domain (amino acids 319—360) are highlighted, along with nuclear localization sequences (amino acids 316—325) and a putative DNA damage recognition domain (amino acids 311—393). The location of p53 mutations are shown relative to these functionally important p53 domains. Numbers above and below the p53 diagram represent numbers of missense and non-missense (deletion, truncation, or frameshift) mutations, respectively. With the exception of two cell lines (NCI-H226 and SN12C), p53 mutations were found clustered into the central conserved DNA binding domain of p53. p53 domain structure was redrawn with modifications from Greenblatt et al. (33).
Table 3 Basal level expression of p53 protein in the NCI cell screen lines

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<th>Basal level of p53 protein</th>
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<tr>
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</tr>
<tr>
<td>mutant</td>
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<tr>
<td>wt/mutant</td>
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<tr>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
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\(^a\) The basal levels of p53 in each line were classified into two groups according to the level of p53 expression in WMN cells (wild-type p53). Lower negligible basal p53 level, <2-fold that seen in WMN cells; high basal p53 level, ≥2-fold that seen in WMN cells.

The basal levels of p53 in each line were classified into two groups according to the level of p53 expression in WMN cells (wild-type p53). Lower negligible basal p53 level, <2-fold that seen in WMN cells; high basal p53 level, ≥2-fold that seen in WMN cells.

14 lines contained wild-type p53. Twenty-two of the cell lines (58%) showed minimal G1 arrest at 12.6 Gy, and all but 2 (9%) contained mutant p53. Only 2 lines (5%) showed intermediate G1 arrest at 12.6 Gy, and both of these contained mutant p53.

The yeast growth assay suggested that RPMI8226 cells contained transcriptionally active p53 despite a mutation at codon 285 (E/L). These cells, however, failed to arrest in G1 following γ-irradiation (Fig. 3), suggesting that the p53 pathway was defective in this line. The CNS cell line SN12C, which harbored a p53 mutation outside of the sequence specific DNA binding domain of p53 (codon 336: E to stop), also failed to arrest in G1. This result was consistent with the lack of activity of p53 cDNA from SN12C cells in the yeast survival assay (Table 1).

Effect of γ-Irradiation on CIP1/WAF1, GADD45, and MDM2 mRNA Levels and Relationship to p53 Status. The functional status of the p53 pathway in the 60 cell lines of the NCI screen was also assessed by measuring the γ-ray inducibility of three p53-regulated genes, CIP1/WAF1, GADD45, and MDM2. Exponentially growing cells were irradiated with 20 Gy of γ-rays, and 4 h later, polyadenylated mRNA was extracted from each line and subjected to a quantitative dot-blot hybridization assay (30, 31). The degree of induction of each transcript was related to basal expression levels, and equivalent mRNA loadings were ensured by also assaying for the constitutively expressed housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (see "Materials and Methods"). The cell lines were categorized into three groups based on the magnitude of mRNA induction for each transcript. Class 1 cells were those that exhibited strong mRNA induction (>4-fold above basal levels), and such a response was interpreted as indicative of a functionally responsive p53 pathway in such cells. Class 3 cells were those that showed minimal or no mRNA...
Fig. 3. Degree of G1 arrest induced by 6.3 or 12.6 Gy of γ-rays in 48 of the NCI cell screen lines. A, percentage of the original G1 population that remained in G1 for 17 h following exposure to 6.3 or 12.6 Gy of γ-irradiation. Cell cycle distribution was quantitated using flow cytometry as described in “Materials and Methods.” Measurements shown were made in the presence of the mitotic inhibitor nocodazole to ensure that cells that broke through the G2 checkpoint did not reenter G1. The individual cell lines have been grouped according to the tissue of origin for each line. B, ranked order of G1 arrest induced by 6.3 or 12.6 Gy of γ-irradiation. G1 arrest responses were grouped into three classes: class 1 cells showed strong G1 arrest (>20% of the original G1 population); class 2 cells showed intermediate G1 arrest (10–20% of the original G1 population); and class 3 cells showed weak or no G1 arrest (<10% of original G1 population). Twelve lines were deemed to grow too slowly for the G1 arrest test and were not included in the analysis (Table 1). Arrows, wild-type p53 lines showing weak or no G1 arrest.
induction (<2-fold above basal levels), and such a response was interpreted as a sign of p53 dysfunction. Class 2 cells showed intermediate mRNA induction levels (between 2-fold and 4-fold).

Of the 60 cell lines assayed for γ-irradiation inducibility of CIP1/WAF1 mRNA, 18 lines (30%) showed strong induction of this transcript (class 1), and with the exception of SNB19 cells (258 E/K) all of these lines contained wild-type p53 sequence (Fig. 4). The only wild-type p53 line that did not exhibit CIP1/WAF1 mRNA induction was A498. The slowly growing nature of this line (doubling time, 67 h) prevented accurate measurement of G1 arrest by flow cytometry. Thirty-nine cell lines (65%) failed to induce CIP1/WAF1 mRNA following exposure to 20 Gy of γ-rays (class 3), and 36 of these lines (92%) contained mutant p53 (Fig. 4). The two remaining lines that lacked CIP1/WAF1 mRNA induction were HCT15 cells, which contained a heterozygous p53 mutation, and BT549 cells, which have previously been reported to harbor mutant p53 (18).

Strong γ-irradiation induction of GADD45 mRNA (class 1) was observed in six cell lines (10%), all of which contained wild-type p53 (Fig. 5). Eight lines induced GADD45 mRNA to levels between 2-fold and 4-fold, and p53 sequencing data on six of these lines revealed wild-type p53 sequence (Table 1). Taken together, 11 of the 18 wild-type p53 cell lines (61%) assayed demonstrated moderate to strong (>2-fold) induction of GADD45 mRNA. The degree of GADD45 mRNA induction was therefore less precise in pinpointing wild-type p53 cell lines compared to similar assessments on the level of CIP1/WAF1 mRNA induction. This appeared to be due to four melanoma (UACC257, SKMEL5, UACC52, and LOX) and two renal cell lines (CAKI and A498), all of which contained wild-type p53 but showed limited or no induction of GADD45 mRNA following γ-irradiation. Lack of GADD45 mRNA induction occurred despite the strong induction of CIP1/WAF1 mRNA in five of these cell lines (compare Figs. 4 and 5; see Ref. 37). G1 arrest assays performed on three of these lines (UACC52, SKMEL5, and LOX) also showed moderate to strong induction of G1 arrest (Fig. 4). Our results indicated that a possible defect(s) in these lines resides at the level of GADD45 induction (37). Forty-six cell lines failed to show induction of GADD45 mRNA (class 3) following γ-irradiation; of these, 39 cell lines (85%) contained mutant p53, 6 lines (13%) contained wild-type p53, and one cell line, HCT15, was heterozygous for p53 mutation.

With the exception of one cell line (ACHN), the degree of MDM2 mRNA induction observed in cells with wild-type p53 was generally weaker than that observed for either CIP1/WAF1 or GADD45 (compare the Y-axis values of Figs. 4, 5, and 6). Of the 12 cell lines that induced MDM2 mRNA above 2-fold, 9 (75%) were wild-type for p53, and 3 lines (25%) were mutant for p53 (Fig. 6). The mutant p53 lines that exhibited moderate induction of MDM2 mRNA were SF295, CCRFCEM, and HL60 cells. Although the remaining nine wild-type p53 lines assayed showed relatively weak or no MDM2 induction (<2-fold), the majority of these lines (six of nine) showed MDM2 mRNA induction of between 1.5-fold and 2-fold. A comparable level of MDM2 mRNA induction was seen in only 1 of the remaining 37 mutant p53 lines tested. These results illustrate that when the level of MDM2 mRNA induction was set at 1.5-fold or above, transcriptional activation of MDM2 can pinpoint 83% of the wild-type p53 cell lines tested.

Correlations between the p53 cDNA Status of the 60 Cancer Cell Lines of the NCI Screen and the Growth-Inhibitory Potency of Bleomycin, 5-Fluorouracil, and Cisplatin. We first investigated whether p53 cDNA status could explain differences in the sensitivity of the 60 cancer lines in the NCI screen to bleomycin, 5-fluorouracil, and cisplatin. Fig. 7 shows the dose of each of these agents required to inhibit growth of the cells by 50% with respect to the vehicle-treated control population (GI50). Data on the sensitivity of the cell lines to each agent are plotted according to the status of p53 cDNA sequence (Table 1). Eighteen wild-type p53 and 39 mutant p53 cell lines were assessed in each case, and each point on the graphs in Fig. 7 represents the averaged GI50 from multiple experiments for an individual cell line treated with that agent. The solid bars (Fig. 7) shown within each plot represent the median response of each group of cell lines. We found that when taken together as a group, the mutant p53 cell lines tended to be less sensitive to bleomycin-, 5-fluorouracil-, and cisplatin-induced growth inhibition than the wild-type p53 cell lines. The median GI50 for the group of mutant p53 lines treated with bleomycin was approximately 2.5 μM, compared to approximately 0.25 μM for the group of wild-type p53 cell lines (10-fold difference). The median GI50 for the group of mutant p53 lines treated with 5-fluorouracil was approximately 25 μM, compared to approximately 4 μM for the group of wild-type p53 cell lines (6-fold difference), and the median GI50 for the group of mutant p53 lines treated with cisplatin was approximately 5 μM, compared to 1.5 μM for the group of wild-type p53 cell line (3.3-fold difference).

Included in Fig. 7 are values obtained from the Wilcoxon rank sum test (p value = 0.996–0.999). If the p values derived from this nonparametric test were to be interpreted statistically, then values >0.975 or <0.025 would be required to reject the null hypothesis of equal potencies for the p53 mutant and wild-type lines for any given agent. Because the 60 cell lines do not, however, represent a random sample from a definable underlying population, the p values should be considered simply as a convenient parameter for describing tendencies in the data. For example, a p value of 0 indicates a compound that tended to be more potent in the p53 mutant lines; a p value of 1 indicates a compound that tended to be more potent in the p53 wild-type lines; a p value of 0.5 indicates no trend in either direction. The Wilcoxon rank-sum test supported the observed tendency of the mutant p53 lines to be less sensitive to bleomycin, 5-fluorouracil, and cisplatin compared to the wild-type p53 cell lines (bleomycin, p value = 0.999; 5-fluorouracil, p value = 0.996; cisplatin, p value = 0.998).

In the case of bleomycin or 5-fluorouracil, only 2 of the 18 wild-type p53 lines exhibited less sensitivity to these agents than the median for the mutant p53 cell lines, and only 5 of 39 mutant p53 lines (13%) exhibited greater sensitivity to these agents than the median value for the wild-type p53 cell lines (Fig. 7). In the case of cisplatin, only 1 of the 18 wild-type p53 lines exhibited less sensitivity to cisplatin than the median for the mutant p53 cell lines, and only 7 of 39 mutant p53 lines (18%) exhibited greater sensitivity to cisplatin than the median for the wild-type p53 cell lines (Fig. 7). Taken together, these findings support an observed trend in the data that suggested that when taken as a group, the mutant p53 cell lines tended, in this assay, to be less sensitive to bleomycin-, 5-fluorouracil-, and cisplatin-induced growth inhibition than the wild-type p53 cell lines.

Despite this trend in the data, there was a fair degree of spread in the range of GI50s for either the group of wild-type p53 lines or the mutant p53 lines. This spread could not be accounted for on the basis of differences in the doubling time for each cell line or upon the cell cycle distribution patterns of exponentially growing cells at the start of treatment (Table 1 and data not shown). We investigated whether any additional p53 parameters might help explain the spread of responses within each group to the drug cisplatin. In the case of the mutant p53 lines, we could not significantly account for this spread on the basis of subdividing the mutant p53 lines according to (a) p53 protein expression levels, (b) missense versus nonmissense mutations, or (c) hot spot versus non-hot spot mutations (data not shown). Subdivision on the basis of the position of the amino acid mutation was not possible due to the small sample size for each mutation (Fig.
Fig. 4. Degree of γ-ray inducibility of CIP1/WAF1 mRNA in 60 lines from the NCI screen. A. exponentially growing cells were exposed to 20 Gy of γ-rays; 4 h later, cells were lysed, and polyadenylated mRNA was extracted from a control or an irradiated sample for each line. CIP1/WAF1 mRNA levels were assayed using a quantitative dot-blot procedure (see "Materials and Methods"). Shown is the degree of CIP1/WAF1 mRNA induction relative to basal levels of this transcript ordered according to tissue type. B. ranked order of CIP1/WAF1 mRNA induction. The degree of CIP1/WAF1 mRNA induction was grouped into three classes: class 1 cells showed strong induction of CIP1/WAF1 mRNA (>4-fold above basal levels); class 2 cells showed intermediate induction of CIP1/WAF1 mRNA (between 2-fold and 4-fold); and class 3 cells showed weak or no induction of CIP1/WAF1 mRNA (<2-fold). The Y axis has been limited to 16-fold mRNA induction above basal levels. Arrows, cell lines showing unusual CIP1/WAF1 mRNA induction responses.
Fig. 5. Degree of γ-ray inducibility of GADD45 mRNA in 60 lines from the NCI screen. A, degree of GADD45 mRNA induction relative to basal levels of this transcript ordered according to tissue type. GADD45 mRNA levels were assayed as described in the legend of Fig. 4 and "Materials and Methods." B, ranked order of GADD45 mRNA induction. The degree of GADD45 mRNA induction was grouped into three classes as described in the legend of Fig. 4 and "Materials and Methods." Arrows, cell lines showing reduced or negligible GADD45 mRNA induction responses despite containing wild-type p53.
Fig. 6. Degree of γ-ray inducibility of MDM2 mRNA in 60 lines from the NCI screen. A, degree of MDM2 mRNA induction relative to basal levels of this transcript, ordered according to tissue type. MDM2 mRNA levels were assayed as described in the legend of Fig. 4 and "Materials and Methods." B, ranked order of MDM2 mRNA induction. The degree of MDM2 mRNA induction was grouped into three classes as described in the legend of Fig. 4 and "Materials and Methods."
In the case of the wild-type p53 lines, we again could not significantly account for the spread in responses to cisplatin on the basis of subdividing the wild-type p53 lines according to (a) the strength of G1 arrest or (b) induction of the p53-regulated genes CIP1/WAF1, GADD45, and MDM2 (data not shown). These findings suggested that factors in addition to the ones we measured contributed to the final variation in the GI50. This was not unexpected, based on the multigenetic nature of human cancer and the presumed variable genetic background of these individual cancer cell lines. A full multivariate analysis using additional molecular characteristics being assessed for the lines might help to explain this spread of responses for cisplatin and the larger set of compounds tested in the NCI screen.

**Correlations between the Complete Set of p53 Status Measurements Recorded on the NCI Screen Cell Lines and the Growth-Inhibitory Potency of 123 Standard Agents.** The complete set of p53 assessments gathered on the NCI screen cell lines were next used to probe a database of growth-inhibitory potencies for 123 standard agents, which included the majority of clinically approved anticancer drugs. These agents make up a standard set of compounds that have been tested against the lines on multiple occasions and for which the mechanism of action of each had previously been assigned (5, 8, 10). The p53 characterization data reported here has been used against the wild-type p53 cDNA status than were the other p53 measurements we recorded (p53 cDNAs in the yeast growth assay, γ-ray induction of CIP1/WAF1 or MDM2, and γ-ray-induced G1 arrest at 12.6 Gy). Indeed, in the case of GADD45 mRNA induction, there were six cell lines (UACC257, SKMEL5, UACC62, CAKI1, A498, and LOXIMVI) that exhibited minimal or no GADD45 mRNA induction following irradiation despite containing wild-type p53 sequence (Fig. 5). Five of these cell lines had robust induction of CIP1/WAF1 mRNA, whereas one (A498) was deficient in CIP1/WAF1 mRNA induction. In the case of G1 arrest, in which we applied a cutoff of >20% G1 arrest for intact p53 function (Fig. 3), only 9 of 16 wild-type p53 lines tested were accurately predicted to be wild-type p53, whereas 14 of the 16 cell lines were accurately predicted to be wild-type p53 at 12.6 Gy.

Correlations uncovered when p53 protein expression levels were used tended to be opposite those obtained with the wild-type p53 cDNA sequence (Fig. 8). This was not unexpected, because the majority of the mutant p53 cell lines analyzed (>70%) overexpressed the mutant p53 protein (Fig. 2).

In contrast to most of the compounds in the four mechanism of action classes described above, we found that the activity of the antimitotic compounds, such as paclitaxel, vincristine, and vinblastine did not correlate with any of the p53 status measurements we made (Fig. 8). By way of example, the individual cell line responses to paclitaxel, grouped according to p53 cDNA status, are shown in Fig. 9. The results suggest that for the majority of agents within the antimitotic class, p53 status was not a major predictor of growth-inhibitory activity in the NCI screening assay.

**DISCUSSION**

This study reports (a) the status of the p53 tumor suppressor pathway in 60 cancer cell lines used in the NCI anticancer drug screen, and (b) relationships between p53 status and the activity of 123 standard agents, the mechanisms of drug action of which have previously been assigned (5, 8, 10). The p53 characterization data reported here has also made possible a recent information-intensive approach to the molecular pharmacology of cancer as it relates to the p53 tumor suppressor pathway in the NCI cell screen cell lines (38).

Complete bidirectional p53 cDNA sequencing on 58 of the 60 cell
lines in the NCI anticancer drug screen revealed that 18 lines contained wild-type p53 sequence, 39 lines contained only mutant p53, and 1 line was heterozygous for p53 mutation (Table 1; Fig. 1). The overall frequency with which mutant p53 status was observed in the NCI screen cell lines (68%) was similar to that observed in cell lines derived from Burkitt’s lymphoma (39), head and neck cancer (40), colon cancer (41) and lung cancer (42). In each case, however, the frequency with which mutant p53 status was observed in the cancer cell lines was somewhat higher than that found in primary tumor samples (Ref. 33 and references therein). Consistent with the relative infrequency of p53 mutations in primary melanoma and renal cancer (Ref. 33 and references therein), we found that the melanoma and renal cell line panels in the NCI screen had a relatively higher number of wild-type p53 lines (9 wild-type p53 lines of 16 (56%) compared to the remaining tissue panels [9 wild-type p53 lines of 41 (22%)].

The majority of p53 sequence mutations observed in the NCI screen lines were missense mutations, and approximately 73% of these mutations occurred at G:C base pairs (Table 1; Fig. 1). The most frequent base alterations were G/A (41% of cases) and G/T (12%) replacements (Fig. 1), and the frequency with which these changes occurred was similar to that observed in an analysis of 2567 primary tumor samples (G/A, 41%; G/T, 17%; Ref. 33). This was also the case for deletion/insertion mutations (NCI screen lines, 17%; primary tumor samples, 13%; Ref. 33). In agreement with previous studies (Refs. 18 and 33 and references therein), the majority of p53 mutations were clustered into the central evolutionarily conserved DNA binding domain of p53. Within this region, a number of hot spot p53 mutations were observed, including mutations at amino acids 248 and 273 (which do not significantly affect p53 structure; Ref. 43 and references therein). In the crystal structure of the p53-DNA complex, these amino acids make contacts with the DNA (43). Two cell lines had mutations at arginine-175, and two lines had mutations at histidine-179 (one of the four zinc binding sites in p53). Both of these residues appear to be critical in stabilizing p53 conformation, and mutations at these sites have previously been associated with a denatured p53 structure (Ref. 43 and references therein).

The yeast-based survival assay provided a means of directly assessing the functional significance of each p53 mutation (28). This assay selects for a transcriptionally competent p53 sequence (see “Materials and Methods”). Of the 38 mutant p53 cell lines assayed, 36 (95%) lacked transcriptional activity in the yeast assay. The two mutant p53 lines deemed wild-type p53 in the yeast assay (RPM18226, 285 E/L; SKMEL2, 245 G/S) failed to exhibit γ-ray-induced G1 arrest and/or γ-ray-induced expression of CIP1/WAF1 or

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**Fig. 8. Wilcoxon P matrix relating the activity of 123 agents with assigned mechanism of drug action to p53 status measurements.** P values derived from the Wilcoxon rank-sum test indicate the apparent dependence of the activity of a drug on each p53-pathway component. The values have been color coded according to the scale shown in the figure. For each drug, red indicates higher sensitivity in the group of human cell lines defined as normal p53 according to the following criteria: p53 wild-type sequence; wild-type p53-like activity in the yeast assay; CIP1/WAF1 mRNA induction greater than 2-fold; GADD45 mRNA induction greater than 2-fold; MDM2 mRNA induction greater than 1.5-fold; G1 arrest at 6.3 Gy greater than 20%; and G1 arrest at 12.6 Gy greater than 20%. Cells exhibiting high basal expression of p53 tended to differ in their responses to these compounds compared to cells defined as normal p53. These results are consistent with the high frequency with which mutant p53 sequence was found in cells expressing high basal levels of p53 (see Fig. 2 and Table 3). The proposed mechanism of action of each group of compounds is shown at the top of the figure (the term “alkylating agents” is used broadly and includes platinating agents, such as cisplatin). Some sample compounds from each of these groups have been highlighted along the base of row 8. The scale at the bottom shows the color-coding of Ps, ranging from P = 0 to P = 1.
GADD45 mRNA. The discrepancy between the yeast assay and functional tests in RPMI8226 and SKMEL2 cells might be explainable if the mutations were temperature sensitive, because the lower temperature at which the yeast assay is performed might favor a wild-type p53 conformation (28). Alternatively, these particular mutants may have been false positives in the yeast assay used, or these two cell lines could harbor other defects in the p53 pathway. The yeast assay was successful in determining the heterozygous p53 nature of HCT-15 cells, as well as all of the wild-type p53 cell lines assayed. Overall, the success rate of the yeast assay in determining p53 status was 96%.

Assessment of basal p53 protein levels revealed that 70% of the NCI cell screen lines containing mutant p53 sequence expressed elevated basal levels of the mutant p53 protein. Interestingly, three wild-type p53 lines (OVCAR4, IGROV1, and SF539) also expressed appreciable basal levels of p53. p53 protein determinations were thus not always effective in establishing p53 gene status. Of the NCI screen lines that contained hot spot mutations, all of those with a 248 R/Q (3 lines), 273 R/H (4 lines), or 266 G/E (4 lines) mutation expressed elevated p53 protein levels. Curiously, two cell lines that contained a 248 mutation that changed an arginine to leucine, instead of to glutamine, did not accumulate p53 (Fig. 2). Comparisons between mutations expected to alter p53 conformation (175 R/H and 179 H/R) and those that would not (248 R/Q and 273 R/H) did not reveal a consistent basis for p53 accumulation in these cancer cell lines. Also, KM12 and SKOV3 cells exhibited different p53 levels despite the fact that both contained a 179 H/R mutation that would destabilize p53 structure (Ref. 43 and references therein). These results suggest that other cellular factors may also govern to the accumulation of mutant p53 protein in cells.

A requirement for wild-type p53 sequence in γ-ray-induced G1 arrest in cell lines from the NCI screen supports earlier reports that established the significance of p53 in regulating the G1 checkpoint response to DNA damage (36). We found that only 2 of the 16 wild-type p53 lines tested at 12.6 Gy of γ-rays failed to undergo G1 arrest. Indeed, G1 arrest measurements made at 12.6 Gy were more indicative of p53 cDNA status that those made at 6.3 Gy. Our G1 arrest results were obtained from irradiated cells that were incubated in medium containing the mitotic inhibitor nocodazole. Nocodazole was added to the assay to ensure that any cells that break through the G2 checkpoint were prevented from reentering G1 of the second cell cycle. Thus, the G1 population that was measured 17 h following irradiation and incubation with nocodazole was composed of cells arrested in the first G1. (20, 21).

Assessment of the γ-ray inducibility of mRNA transcribed from the p53-regulated genes CIP1/WAF1, GADD45, and MDM2 revealed, in most cases, an apparent dependence upon wild-type p53 sequence for strong induction of each transcript. Noteworthy exceptions included the curious lack of GADD45 mRNA induction in some of the melanoma and renal cell lines, γ-ray induction of CIP1/WAF1 mRNA in one mutant p53 line, and MDM2 mRNA induction in three mutant p53 lines. Lack of induction of GADD45 mRNA in some of the wild-type p53 melanoma and renal lines contrasted with the strong induction of CIP1/WAF1 mRNA in 5 of 6 lines. These results suggested that failure of p53 to induce GADD45 mRNA resided at the level of the GADD45 gene or mRNA stability. Sequencing of the third intron region of the GADD45 gene, which contains the p53 binding element, revealed no sequence mutations in this region (37). A further investigation of these and other melanoma and renal cell lines will be necessary to explain this unexpected finding. The level of MDM2 mRNA induction observed in the wild-type p53 lines studied was relatively modest compared to that observed for either CIP1/WAF1 or GADD45 mRNA. Fifteen of the 18 wild-type p53 lines tested exhibited induction of MDM2 mRNA levels above 1.5-fold (Fig. 6). However, so did a number of mutant p53 cell lines, including SF295, CCRFCEM, and HL60 cells. The basis for MDM2 mRNA induction in these mutant p53 lines requires further investigation, as does the finding of one mutant p53 line, SNB75, that showed marked γ-ray inducibility of CIP1/WAF1 mRNA. The latter result, however, was made relative to basal CIP1/WAF1 mRNA levels, which in SNB75 cells were particularly low (data not shown).

Characterization of the integrity of the p53 pathway in the NCI screen lines provided the opportunity to examine the activities of 123 standard agents for which putative action mechanisms had been assigned (5, 8, 10). Most of the clinically approved anticancer drugs were included within this set of compounds. Our goal here was to determine whether p53 status was an important factor in the chemosensitivity of the 60 cell lines in the NCI screening assay. The results shown in Fig. 8 indicated that the majority of clinically active agents, including alkylating agents, antimetabolites, and topoisomerase I and II inhibitors, tended, in this assay system, to exhibit more growth suppression in the lines with normal p53 status. This was the case whether the analysis was based on p53 cDNA sequence or on most of the other factors that indicated an intact p53 pathway. In contrast, cells with elevated p53 protein levels tended to be less sensitive to the majority of these same agents. This finding was consistent with the high frequency with which mutant p53 gene status was observed in lines overexpressing p53 (Fig. 2).

Given the above findings, we have recently gone on to identify compounds for further investigation on the basis that their activity in this primary screen tended not to correlate with p53 status or tended toward greater activity in the lines with mutant p53 (Ref. 38 and data not shown). Although agents of the latter type were not uncovered among the 123 standard agents tested in the present report, a series of compounds that clearly differed from the majority of clinical drugs was the antitubulin series, which includes paclitaxel and vincristine. For these drugs, there was no correlation between p53 status and chemosensitivity in the NCI screening assay. We uncovered similar findings in recent studies on a series of Burkitt’s lymphoma cell lines and with a number of isogenic systems based on cancer cells with intact or disrupted p53 function.3 In contrast to the above findings,

3 S. Fan, B. Cherney, and P. M. O’Connor. Disruption of p53 function in immortalized human cells does not affect survival or apoptosis following paclitaxel or vincristine treatment, submitted for publication.
however, it has been reported that p53 disruption sensitizes normal fibroblasts to paclitaxel (44). Although further studies are necessary, it is possible that the paclitaxel sensitization observed in normal cells lacking p53 function may not always extend to cancer cell lines. The differential cisplatin sensitivity of the wild-type and mutant p53 lines in the NCI screen paralleled our earlier results in human lymphoma cells (21). In these previous studies, we correlated the reduced cisplatin sensitivity of the mutant p53 lines with an evasion of p53-mediated apoptosis (21). Investigations into the susceptibility of the NCI cell screen to p53-dependent apoptosis might help extend our observations described in this report. We and others have found that disruption of p53 function in breast cancer MCF-7 cells and other cell lines can actually cause cisplatin sensitization (19, 25, 26). A major difference between MCF-7 cells and those of lymphoid origin, however, is their susceptibility to p53-mediated apoptosis. In the case of lymphoid cells, activation of p53 has been associated with apoptosis (21), whereas MCF-7 cells respond to p53 activation by stably arresting in G1 and G2 (19). Cisplatin sensitization might be more clearly observed when cell types not inherently prone to p53-induced apoptosis have been disrupted for p53 function. Given that the majority of the mutant p53 lines in the NCI screen tended to be less sensitive to cisplatin than the wild-type p53 lines, it seems that the responses of the NCI lines more closely resembled the responses observed in human lymphoma cells (21). Further studies will be necessary to investigate whether apoptosis is an important determinant of the GI50 recorded for the wild-type p53 cell lines in the NCI cell screen.

The current NCI anticancer drug screen provides a short-term assay of chemosensitivity defined through growth inhibition. The assay screens approximately 10,000 compounds per year, and to date, over 60,000 agents have been tested against these lines. This assay cannot, with certainty, distinguish cytotoxicity from cytostasis, and agents selected from this primary screen based on interesting activity profiles against the lines therefore need to be tested in additional assays for cytotoxicity. Nonetheless, the results obtained in this short-term assay have already yielded useful insights into mechanisms of drug action, P-glycoprotein substrates, and agents preferentially active in cells with activated Ras or nm23 expression differences (5, 8, 10—15, 32). In each case, agents and correlations have been discovered from these databases that might not have been found otherwise.

Our characterization of the p53 tumor suppressor pathway in the NCI screen permitted the large database of compounds tested against these cell lines to be searched for agents that might have preferential activity against cells with defective p53 function. The initial findings of these studies were briefly described in a recent report (38). Verification that any such lead compounds derived from this (38) or subsequent analyses actually behave differently in relation to p53 status will clearly be necessary. This verification process will involve examination of compounds in isogenic systems consisting of parental wild-type p53 lines and derivative cell types lacking p53 function. This is possible through transfection with either a dominant-negative mutant p53 gene or the human papillomavirus type-16 E6 gene (19, 26). Other characteristics will have to be taken into account when appropriate cell lines for transfection are chosen. For example, cell types representative of the two contrasting arms of the p53 response pathway, apoptosis and stable cell cycle arrest, will need to be tested in such studies. Longer-term assays of chemosensitivity, such as 6-day survival assays and clonogenic assays, as well as in vivo studies, are intended to confirm that lead compounds do indeed exhibit preferential cytotoxicity against cells with defective p53 function. The present report provides extensive information regarding the status of the p53 pathway in cell lines of the NCI anticancer drug screen. This information should prove useful to researchers investigating fundamental aspects of p53 biology and pharmacology. A summary of these findings and links to other molecular characteristics gathered on the NCI screen cell lines can be accessed through the World Wide Web at http://epnws1.ncifcrf.gov:2345/dis3d/dtp.html. This information on p53 also enabled us to investigate the growth-inhibitory properties of a series of 123 standard agents previously characterized according to mechanism of drug action in the NCI screen. Our investigations revealed that cell lines with defective p53 status tended, in this assay system, to be less sensitive to the majority of alkylating agents, antimetabolites, and topoisomerase I and II inhibitors. No such relationship was seen for the antimetabolic agents, such as paclitaxel and vincristine. Our characterization of p53 in these cell lines is enabling a large scale analysis of the more than 60,000 compounds tested in this primary screen. The next goal is to discover novel agents that act independently of p53 function or that might exploit defective p53 function as a means of preferential toxicity.

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p53 AND CHEMOSensitivity


Characterization of the $p53$ Tumor Suppressor Pathway in Cell Lines of the National Cancer Institute Anticancer Drug Screen and Correlations with the Growth-Inhibitory Potency of 123 Anticancer Agents

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