Disruption of p53 Function in Immortalized Human Cells Does Not Affect Survival or Apoptosis after Taxol or Vincristine Treatment

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ABSTRACT

In the present study, we report our findings on the impact of p53 disruption on the sensitivity of human cell lines to the antimitotic agents Taxol and vincristine. Comparisons of cell survival and apoptosis were made with γ-irradiation and, in some cases, several other DNA-damaging chemotherapeutic agents. Studies in eight Burkitt's lymphoma and lymphoblastoid cell lines (four wild-type p53 and four mutant p53 cell lines) revealed that the DNA-damaging agents assayed tended to exhibit less growth inhibition in the mutant p53 cell lines compared to the wild-type p53 cell lines. In contrast, no significant correlation was apparent between p53 gene status and the growth-inhibitory potency of Taxol or vincristine in these eight cell lines. We also found that contrary to γ-irradiation, Taxol and vincristine could induce apoptosis in lymphoma cell lines harboring p53 mutations. These observations were explored further in lymphoblastoid VDSO cells (wild-type p53) from a normal individual and stably transfected VDSO derivatives lacking p53 function due to expression of the human papillomavirus type-16 E6 gene. We found that p53 disruption in VDSO/E6 cells blocked γ-ray-induced apoptosis and afforded a survival advantage to VDSO/E6 cells compared to control-transfected cells. In contrast, p53 disruption did not affect Taxol- or vincristine-induced apoptosis or survival in VDSO cells. The effect of p53 disruption on Taxol sensitivity was explored further in the breast carcinoma MCF-7 and colon carcinoma HCT-116 cell lines that had been stably transfected with either the human papillomavirus type-16 E6 gene or a dominant-negative mutant p53 gene. Again, in these cell model systems, we found that p53 disruption did not affect the growth-inhibitory potency of Taxol. Taken together, our results suggest that p53 status is not a dominant factor in the mechanism by which antimitotic agents induce apoptosis and reduce survival in immortalized human cell lines.

INTRODUCTION

The p53 tumor suppressor gene regulates a complex array of cellular responses to DNA damage, including cell cycle arrest in G1 phase, apoptosis, and/or some aspects of DNA repair (1–3). These responses are lost in the majority of human tumor cell lines and many human primary tumors due to inactivating mutations in the p53 gene (4, 5). The cellular consequences of these loss-of-function mutations in p53 are profound and include genomic instability and, in certain cell types, reduced apoptotic potential (Refs. 2 and 3 and references therein). Alterations in the p53 pathway can also impact the sensitivity of mammalian cells to ionizing radiation and at least some DNA-damaging chemotherapeutic agents. In the case of those cell types that are susceptible to p53-induced apoptosis, disruption of p53 function either by mutation or disruption of the p53 gene or by stable transfection of cells with dominant-negative acting factors including mutant p53 or the human HPV-E6 oncogene, can reduce the degree of apoptosis normally induced by DNA-damaging agents (6–13). In cell types that are not inherently prone to p53-mediated apoptosis but instead undergo a stable cell cycle arrest after p53 activation, the situation is more complex (14). In the case of ionizing radiation, disruption of p53 function in such cell types often does not affect clonogenic survival (11, 15–17). However, for certain DNA-damaging chemotherapeutic agents, i.e., CDDP and nitrogen mustard, and UV radiation, there is actually a detrimental effect on colony formation after p53 disruption (17–20). Taken together, such studies have highlighted the importance of cellular context in studying the role of the p53 tumor suppressor pathway in radiosensitivity and chemosensitivity.

Although the impact of p53 disruption on the sensitivity of mammalian cells to DNA-damaging agents has received much attention, the situation for other classes of chemotherapeutic agents still remains relatively unexplored. One class of agents that has received prominent clinical utilization is the antimitotic agents, such as Taxol and vincristine. Such agents act by interfering with the dynamics of microtubule assembly or disassembly and arrest cells in a “pseudo-metaphase-like” state (Refs. 21 and 22 and references therein). In the present study, we investigated whether p53-defective immortalized cell lines differ in their sensitivity to Taxol and another antimitotic agent, vincris-
p53 and Chemosensitivity

We report that p53 disruption in human lymphoid cells tended to reduce the sensitivity of such cells to DNA-damaging agents, but in contrast, no significant impact of p53 disruption was observed on the sensitivity of these cells to either Taxol or some of their characteristics are shown in Table 1. Comparisons of cell survival and apoptosis were made with γ-irradiation, and in some cases, several other DNA-damaging agents.

We report that p53 disruption in human lymphoid cells tended to reduce the sensitivity of such cells to DNA-damaging agents, but in contrast, no significant impact of p53 disruption was observed on the sensitivity of these cells to either Taxol or vincristine. Also, in contrast to γ-irradiation, both antimitotic agents were capable of inducing apoptosis independent of p53 functional status in cells of lymphoid origin. Our survival results with Taxol and vincristine were confirmed in the breast carcinoma MCF-7 cell line and the colon carcinoma HCT-116 cell line in which p53 function was disrupted by stable transfection with the HPV-E6 oncogene or a dominant-negative mutant p53 transgene.

**MATERIALS AND METHODS**

**Cell Culture and Stable Transfection.** The molecular characteristics and status of the p53 gene in the Burkitt’s lymphoma cell lines used in this study have been described in detail in Table 1 and elsewhere (9, 18). The cells were routinely maintained in RPMI 1640 (Mediatech, Washington, D.C.) containing 15% fetal bovine serum (Intergen), 2 m M L-glutamine, 50 units of penicillin, and 50 μg/ml streptomycin (Mediatech). The breast carcinoma MCF-7 and colon carcinoma HCT-116 cell lines were cultured in the same medium containing 5% fetal bovine serum. For stable transfection, 1 × 10⁶ exponentially growing VDSO cells were transfected with control pCMV vector or the same vector encoding the HPV-E6 gene [graciously provided by Dr. Kathleen Cho (Johns Hopkins, Baltimore, MD)]. MCF-7 cells were stably transfected with either the HPV-E6 gene or a 143 V/A mutant p53 transgene, and HCT-116 cells were stably transfected with either the HPV-E6 gene or a 248 R/W mutant p53 transgene. The mutant p53 vectors were graciously provided by Dr. Bert Vogelstein (Johns Hopkins). Transfections were carried out using LipofectAMINE transfection reagent (Life Technologies, Inc.). The pCMV plasmids also contained a neomycin (G418) gene that allowed G418-resistant colonies to be selected 14–21 days after transfection. After expansion, colonies demonstrating a lack of γ-ray-induced G₁ arrest and a lack of p53 or p21 protein accumulation were selected for survival studies. The generation and characterization of p53-disrupted MCF-7 and HCT-116 cell lines have been described previously (11, 17, 18). Survival was determined

**Table 1** Status of the p53 gene and pathway in cell lines used in the present studies

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>Transfection</th>
<th>EBV</th>
<th>p53 gene status</th>
<th>Doubling time (h)</th>
<th>G₁ arrest</th>
<th>G₂ arrest</th>
<th>p21 induction</th>
<th>Apoptosis</th>
</tr>
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<tr>
<td>NL2</td>
<td>Lymphoblastoid</td>
<td>+</td>
<td>wt</td>
<td>+</td>
<td>25</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>FWA</td>
<td>Lymphoblastoid</td>
<td>+</td>
<td>wt</td>
<td>+</td>
<td>25</td>
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<td>+</td>
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<td>VDSO/parental</td>
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<td>+</td>
<td>wt</td>
<td>+</td>
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<td>MCF-7/parental</td>
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<tr>
<td>HCT-116/parental</td>
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* A cells were stably transfected with empty vector (pCMV), vector containing the HPV-E6 gene, or a mutant-p53 transgene (MCF-7, the mutant p53 transgene was 143 V/A; HCT-116, the mutant p53 transgene was 248 R/W; Refs. 17 and 18 and this study).  
* Ref. 9 and this study.  
* p53 sequence was determined by a combination of single-strand conformational polymorphism and/or cDNA sequencing (Refs. 9, 11, 17 and 18).  
* G₁ and G₂ arrest were determined by flow cytometry approximately 16 h after exposure to 6.3 or 12.6 Gy of γ-rays (Refs. 9, 17, and 18 and this study).  
* protein levels were determined 4 h after 6.3 Gy of γ-rays by Western blotting (Refs. 11, 17, 18 and this study).  
* Apoptosis was determined through agarose gel electrophoresis of genomic DNA and/or morphological assessments made 48 h after 12.6 Gy of γ-rays (Refs. 11, 17, 18, and 24 and this study).  
* wt, wild-type p53; Mu, mutant p53.  
* G₁ arrest after exposure to γ-irradiation was transient (Ref. 17).
using growth inhibition assays and/or MTT assays as described previously (9, 11).

**Irradiation and Drug Treatment.** Irradiation was performed using a 137Cs source delivering γ-rays at a dose rate of 3.46 Gy/min. VP16 and CDDP were obtained from the NCI. VP16 was prepared in DMSO. CDDP was dissolved in PBS (pH 7.4). Cells were generally irradiated at room temperature or treated with DNA-damaging agents for 1 h at 37°C. Treatment with vincristine or Taxol was for 24 h.

**Flow Cytometry.** Samples were prepared for flow cytometry as described previously (11). Briefly, cells were fixed in ice-cold 70% ethanol, washed with PBS, treated with RNase (Sigma; 500 units/ml) at 37°C for 15 min, and stained with propidium iodide (Sigma; 50 μg/ml). Cell cycle analysis was performed using a Becton Dickinson fluorescence-activated cell analyzer. Fifteen thousand cells were analyzed for each point, and quantification of cell cycle distribution was performed using the Sum of Broadened Rectangles (SOBR) model program provided by the manufacturer.

**Western Blot Analysis.** Samples were lysed on ice for 30 min in 1% NP40 prepared in PBS that contained 10 μg/ml leupeptin, 10 μg/ml aprotinin, 2 mM 4-(2-aminoethyl)benzene-sulfonyl fluoride, 1 mM sodium o-vanadate, 10 mM sodium fluoride, and 5 mM NaPP. Soluble protein was then boiled for 5 min in a SDS-loading buffer, and 100 μg of total cell protein were loaded onto 15% SDS-polyacrylamide gels. Proteins were then transferred electrophoretically to Immobilon membranes (Millipore, Bedford, MA) and then blocked for 30 min in 5% nonfat milk at room temperature. Immunodetection of p53 protein was performed with a monoclonal pAb1801 antibody (Oncogene Science), and for p21Waf1/Cip1 determination, a polyclonal antibody (PharMingen) was used. Antibody reaction was revealed using enhanced chemiluminescence (Amersham, Arlington Heights, IL).

**RESULTS**

**Human Lymphoma Cell Lines with Mutant p53 Gene Status Tend To Be Less Sensitive to DNA-damaging Agents but not to Antimitotic Agents.** We initially studied the effects of several anticancer agents on the survival of a series of Burkitt’s lymphoma and lymphoblastoid cell lines with either wild-type or mutant p53 gene status (Table 1). These cell lines have previously been characterized in terms of p53 gene status and in regard to the integrity of the p53 pathway after exposure to γ-irradiation (9, 11, 23). Table 1 shows that in contrast to the wild-type p53 lines, the mutant p53 cell lines failed to undergo G1 arrest or apoptosis after γ-irradiation, and this stimuli also failed to induce the p53-regulated gene product p21Waf1/Cip1 in the mutant p53 cell lines. These mutant p53 cell lines were also deficient in γ-ray induction of several other p53-regulated genes (23–25). When taken together, these results provided evidence of the integrity of the p53 pathway in the eight lymphoid cell lines chosen to assess radiosensitivity and chemosensitivity. Consistent with the view that p53 function is not essential for the activation of the G2 checkpoint (9), γ-ray-induced G2 arrest was observed in all of the cell lines tested (Table 1).

In agreement with earlier studies, we found that the mutant p53 Burkitt’s lymphoma cell lines tended to be less sensitive to the growth-inhibitory properties of several DNA-damaging agents compared to the lymphoid cell lines with wild-type p53 status (Table 2; Refs. 9 and 11). A Wilcoxon rank-sum test with normal approximation was applied to the individual cell line responses. In the cases of γ-irradiation, the topoisomerase II inhibitor, VP16, and the two DNA cross-linking agents, nitrogen mustard and CDDP, there was a significant difference in the sensitivity of the group of wild-type cell lines compared to the lymphoid cell lines with wild-type p53 status (Table 2; Refs. 9 and 11). A Wilcoxon rank-sum test with normal approximation was applied to the individual cell line responses. In the cases of γ-irradiation, the topoisomerase II inhibitor, VP16, and the two DNA cross-linking agents, nitrogen mustard and CDDP, there was a significant difference in the sensitivity of the group of wild-type cell lines compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2). In contrast to these DNA-damaging agents, however, we found no significant difference in the sensitivity of the group of wild-type p53 cell lines to the antimitotic agents Taxol or vincristine compared to the group of mutant p53 cell lines (Table 2).

**Table 2** Relationships between p53 status and sensitivity of eight lymphoid cell lines to several anticancer agents

<table>
<thead>
<tr>
<th>Cell line</th>
<th>p53 status</th>
<th>γ-Rays (Gy)</th>
<th>VP16 (μM)</th>
<th>Nitrogen mustard (μM)</th>
<th>CDDP (μM)</th>
<th>Taxol (nm)</th>
<th>Vincristine (nm)</th>
</tr>
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<tr>
<td>FWL</td>
<td>Wild-type</td>
<td>0.7</td>
<td>3.6</td>
<td>0.3</td>
<td>1.5</td>
<td>0.9</td>
<td>80</td>
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<tr>
<td>NL2</td>
<td>Wild-type</td>
<td>1.3</td>
<td>2.6</td>
<td>0.24</td>
<td>0.8</td>
<td>7</td>
<td>9</td>
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<tr>
<td>WMN</td>
<td>Wild-type</td>
<td>0.6</td>
<td>2.9</td>
<td>0.27</td>
<td>1.3</td>
<td>9</td>
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<tr>
<td>AG876</td>
<td>Wild-type</td>
<td>1.1</td>
<td>1.6</td>
<td>0.39</td>
<td>0.3</td>
<td>0.85</td>
<td>70</td>
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<tr>
<td>CA46</td>
<td>Mutant</td>
<td>2.7</td>
<td>3.3</td>
<td>1</td>
<td>3.9</td>
<td>0.8</td>
<td>60</td>
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<tr>
<td>JD38</td>
<td>Mutant</td>
<td>2.3</td>
<td>5.5</td>
<td>0.58</td>
<td>2.2</td>
<td>0.9</td>
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<td>SG588</td>
<td>Mutant</td>
<td>3.8</td>
<td>7.7</td>
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<td>50</td>
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<td>RAMOS</td>
<td>Mutant</td>
<td>3.4</td>
<td>8.8</td>
<td>0.37</td>
<td>1.1</td>
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<tr>
<td>Median wild-type</td>
<td>0.9</td>
<td>2.75</td>
<td>0.285</td>
<td>1.05</td>
<td>3.95</td>
<td>39.5</td>
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<tr>
<td>Median mutant</td>
<td>3.05</td>
<td>6.6</td>
<td>0.505</td>
<td>2.15</td>
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<tr>
<td>p&lt;sup&gt;6&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Values shown are the average of at least three independent experiments.

<sup>b</sup> Wilcoxon rank-sum test (two-tail) with normal approximation for difference in medians of the two groups of cell lines.
cell lines with either wild-type or mutant p53 gene status. Fig. 1A shows representative data from two cell lines, CA46 (mutant p53) and FWL (wild-type p53; Table 1). These two cell lines exhibited marked differences in their sensitivity to γ-rays, determined through either growth inhibition assays (Table 2) or MTT survival assays (Fig. 1A; Ref. 18). Consistent with our previous observations, we found that γ-rays failed to induce DNA fragmentation in the mutant p53 cell line, CA46, whereas the wild-type p53 cell line, FWL, exhibited a clear DNA fragmentation ladder (Fig. 1B; Refs. 11 and 25). When studies were performed at equitoxic doses of irradiation, we also failed to observe apoptosis in the mutant p53 cell line, CA46 (11). In contrast to γ-rays, we found that Taxol (Fig. 1B) and vincristine (data not shown) were capable of inducing a similar degree of DNA fragmentation in CA46 and FWL cells. Similar results to those shown in Fig. 1 were also obtained with two other cell lines containing mutant p53 (JD38 and Ramos; data not shown). Taken together, these studies suggested that p53 function was not required for Taxol or vincristine to induce apoptosis in human lymphoma cells.

**Disruption of p53 Function in Human Lymphoblastoid VDSO Cells Did Not Affect Taxol Sensitivity or Taxol-induced Apoptosis.** Because cancer cell lines often exhibit multiple genetic defects that could impact interpretations of the role of p53 in chemosensitivity and apoptosis, we decided to conduct additional studies in a lymphoid cell background from a normal individual. Studies were conducted in lymphoblastoid VDSO cells (wild-type p53) and derivatives lacking p53 function due to stable transfection with the HPV-E6 oncogene (Table 1). The E6 gene product binds to p53 and stimulates the
After selection, individual clones were expanded and analyzed. This vector also contained a neomycin resistance gene, allowing for p53 function by Western blotting and flow cytometry. Fig. 2 shows a representative data set for parental VDSO cells and for p53 function by Western blotting and flow cytometry. Fig. 2A, and, as expected for a cell line with disrupted p53 function, VDSO/E6–18 cells exhibited a markedly attenuated G1 arrest response to γ-irradiation compared to parental or control pCMV-transfected cells (Ref. 24; Fig. 2B).

Having established the functional status of the p53 pathway in these VDSO derivatives, we next investigated the sensitivity of these cells to γ-rays, Taxol, and vincristine using a MTT assay (Ref. 18; Fig. 3A). Consistent with results gathered on lymphoid cell lines with mutant p53 status (Table 2), we found that approximately a 2-fold higher dose of γ-rays was required to reduce the survival of p53-disrupted VDSO/E6–18 cells to a similar degree (50% survival) compared to parental (VDSO/ parental) or control-transfected cells (VDSO/CMV-8). The ID50 dose for γ-irradiation was 0.95, 1.1, and 2.1 Gy for VDSO/ parental, VDSO/CMV-8, and VDSO/E6–18, respectively (P < 0.01, Student’s t test). Despite altered radiosensitivity after p53 disruption, we found similar sensitivity profiles among the different VDSO derivatives after treatment with Taxol or vincristine (Fig. 3A). We also made comparisons of radiation- and Taxol-induced apoptosis in VDSO/E6 and control-transfected VDSO/CMV cells using DNA fragmentation assays (Fig. 3B). We found that disruption of p53 function in VDSO cells markedly attenuated radiation-induced apoptosis in this cell background, but again, like the mutant p53 Burkitt’s lymphoma cell lines (Fig. 1B), Taxol-induced apoptosis was not impaired in VDSO cells lacking p53 function (Fig. 3B). Taken together with results shown above for the Burkitt’s lymphoma cell lines, our results suggest that p53 function is not required for Taxol- or vincristine-induced apoptosis in human lymphoid cells.

**DISRUPTION OF p53 FUNCTION IN HUMAN BREAST CANCER**

**MCF-7 CELLS AND COLON CARCINOMA HCT-116 CELLS DID NOT AFFECT TAXOL SENSITIVITY.** To determine whether our results for Taxol were limited to cells of lymphoid origin, we also conducted survival experiments in breast carcinoma MCF-7 and colon carcinoma HCT-116 cells. These cell lines harbor wild-type p53 genes, and derivatives lacking p53 function were generated through stable transfection with the HPV-E6 oncogene or a mutant p53 transgene (Table 1; Refs. 17 and 18). In the case of MCF-7 cells, we used cells that had been stably transfected with the dominant-negative 143 V/A mutant p53 gene, and for HCT-116 cells, we used cells that had been stably transfected with the dominant-negative 248 R/W mutant p53 gene. We found that p53 disruption in both the MCF-7 and HCT-116 cell backgrounds, like that in VDSO cells (Fig. 3), did not affect the sensitivity of these cells to Taxol (Fig. 4) or vincristine (Ref. 17; data not shown).

**DISCUSSION**

Our results show that Burkitt’s lymphoma cell lines with a mutant p53 gene status do not significantly differ in their sensitivity to the antimitotic agents Taxol or vincristine relative to lymphoid cells with wild-type p53 status. These observations
contrasted with those uncovered for DNA-damaging agents, which tended to be less effective at inducing growth inhibition in the mutant p53 lymphoma cell lines compared to the wild-type cell lines. Similar results were obtained in an engineered isogenic cell system based on the wild-type p53 lymphoblastoid cell line, VDSO. Results in VDSO cells suggested that lack of a correlation between Taxol- and vincristine-induced cell killing was not due to variations in the genetic backgrounds of the individual lymphoma cell lines analyzed. We also found that in contrast to γ-irradiation, p53 function was not required for Taxol-induced (see Figs. 1 and 3) or vincristine-induced (data not shown) apoptosis in lymphoid cells. Our survival results were not limited to cells of lymphoid origin, because disruption of p53 function in two well-characterized epithelial cell systems (MCF-7 and HCT-116 cells; Refs. 17 and 18) also failed to reveal a correlation between Taxol-induced cytotoxicity and p53 functional status.

Our findings in this report agree with the recent studies we conducted in the NCI anticancer drug screen (3, 29). Characterization of the p53 pathway in the 60 cell lines of this screen enabled us to search for correlations with the growth-inhibitory potency of 123 standard agents that have been tested in this assay system on multiple occasions and for which a mechanism of drug action had previously been assigned. We found that the mutant p53 cell lines in this assay tended, on average, to be less sensitive than the wild-type p53 lines to the majority of alkylating agents, DNA/RNA antimetabolites, topoisomerase I inhibitors, and topoisomerase II inhibitors tested. Thus, results gathered for DNA-damaging agents in the lymphoid cell lines assayed in present study agree with our observations in the NCI screen (3, 29). A class of agents that differed in the NCI screen from the aforementioned anticancer agents, however, was the antimitotic agents such as Taxol and vincristine. As in the present study, p53 status did not seem to be a dominant indicator.
of growth-inhibitory potency of the antimitotic agents in the NCI screen (3, 29). Our present study complements our earlier studies in the NCI screen and extends beyond these earlier findings by showing that in isogenic cell-based assay systems, p53 disruption does not markedly affect the sensitivity of immortalized human cells to the actions of Taxol and vincristine.

In agreement with our findings and while our studies were being written up, Debernardis et al. (30) reported, using a series of wild-type and mutant p53 ovarian cancer cell lines, that p53 status did not correlate with the sensitivity of these cell lines to Taxol. Such results are supported by other recent observations in a mutant p53-transfected derivative of the ovarian cancer cell line A2780, which contains endogenous wild-type p53 (13). Our results support and extend on these observations by showing that p53 disruption in lymphoid, breast, and colon cancer cell lines does not seem to affect Taxol sensitivity or sensitivity to another antimitotic agent, vincristine. Cellular context issues may still apply, however, because Wu and El-Deiry (31) have shown that HPV-E6 can, in certain instances, reduce sensitivity to Taxol. The impact of p53 mutation on the prognosis of patients treated with Taxol is presently being evaluated, and one study in patients with non-small cell lung cancer suggested that in contrast to other chemotherapeutic agents and ionizing radiation, p53 mutations did not predict the response of patients to Taxol therapy (32). Our use of the HPV-E6 gene product to disrupt p53 function was chosen on the basis of previous results from our own and other laboratories and represents a viral gene product routinely used to disrupt p53 function. However, the HPV-E6 gene product can affect other cellular processes, so to help support our contention that p53 disruption does not affect Taxol sensitivity, we included studies conducted on MCF-7 and HCT-116 cells stably transfected with dominant-negative mutant p53.

In summary, we show that in contrast to DNA-damaging agents, p53 disruption in several human immortalized cell-based model systems did not significantly affect the sensitivity of such cells to the antimitotic agents Taxol or vincristine. Furthermore, in contrast to ionizing radiation, apoptosis induced by either Taxol or vincristine was not suppressed by p53 disruption. Our findings in these isogenic cell line systems support recent observations we have made on the chemosensitivity of the 60 cell lines of the NCI anticancer drug screen (14, 29) and highlight the usefulness of isogenic systems in confirmation studies. These and other isogenic systems will also prove useful to analyze lead compounds that are emerging from the NCI screen as candidate agents possibly possessing greater activity in p53-defective cancer cells.

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Disruption of p53 function in immortalized human cells does not affect survival or apoptosis after taxol or vincristine treatment.

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