Expression of *p53* in Cisplatin-resistant Ovarian Cancer Cell Lines: Modulation with the Novel Platinum Analogue (*1R, 2R*-Diaminocyclohexane)(*trans*-diacetato)(dichloro)-platinum(IV)[1]

George S. Hagopian, Gordon B. Mills, Abdul R. Khokhar, Robert C. Bast, Jr., and Zahid H. Siddik[2]

Departments of Gynecologic Oncology [G. S. H.], Molecular Oncology [G. B. M.], and Clinical Investigation [A. R. K., R. C. B., Z. H. S.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

The compound (*1R,2R*-diaminocyclohexane)(*trans*-diacetato)(dichloro)platinum(IV) (DACH-acetato-Pt) is a novel platinum-based antitumor agent with clinical potential against cisplatin-resistant disease that is under development in our laboratory. In view of the central role of the wild-type *p53* tumor suppressor gene in drug-induced apoptosis, we evaluated the cytotoxicity of cisplatin and DACH-acetato-Pt in a panel of cisplatin-resistant ovarian tumor models with differing *p53* status. Cisplatin was relatively more effective against mutant or null *p53* cell lines (continuous drug exposure IC_{50}, 1.2–3.3 μM) than it was against those harboring wild-type *p53* (IC_{50}, 2.8–9.9 μM). In contrast, DACH-acetato-Pt was considerably more active in wild-type *p53* models (IC_{50}, 0.17–1.5 μM) than it was in mutant or null models (IC_{50}, 2.7–11.3 μM). Inactivation of wild-type p53 function in OVCA-429 cells by the human papillomavirus type 16 (HPV 16) E6 plasmid increased resistance to DACH-acetato-Pt by 3–5-fold, which confirmed the drug's dependence on wild-type *p53* for its high cytotoxic potency. Differences between the two platinum agents were also evident in cell cycle studies: cisplatin arrested both wild-type and mutant *p53* cells in G_2-M, whereas DACH-acetato-Pt arrested wild-type *p53* cells in G_1 and mutant *p53* cells in G_2-M. The G_1 arrest by DACH-acetato-Pt was abrogated in HPV 16 E6 transfectant clones of OVCA-429 cells. In agreement with effects on cell cycle progression, a 2-h pulse exposure to low concentrations (≤25 μM) of DACH-acetato-Pt induced marked increases in p53 and $p21^{Waf1/Cip1}$ expression in OVCA-429 cells. Cisplatin, in direct contrast, had no effect on expression of *p53* or $p21^{Waf1/Cip1}$ until the drug concentration was increased to 125 μM. In HPV 16 E6 transfectants of OVCA-429 cells, induction of p53 by the two agents was severely attenuated, and corresponding increases in $p21^{Waf1/Cip1}$ were abrogated. This suggests that $p21^{Waf1/Cip1}$ increases were *p53* dependent. Collectively, the results demonstrate that DACH-acetato-Pt is very distinct from cisplatin. In particular, the greater activity of DACH-acetato-Pt in cisplatin-resistant wild-type *p53* ovarian tumor models can be ascribed to its ability to more efficiently induce p53 protein and activate p53 functions.

INTRODUCTION

Ovarian cancer is the most lethal gynecological malignancy in the United States; in 1997, it was predicted that 26,800 women would develop the disease, and ~14,200 women would die that year (1). Although a number of factors impact on prognosis, including age, histological type and grade, tumor DNA content, extent of disease at presentation, and volume of residual disease, the most important is International Federation of Gynecology and Obstetrics stage (2). The 5-year survival rates for stages I, II, III, and IV are 74, 58, 30, and 19%, respectively (3). Unfortunately, 75% of women present with advanced disease (stages III and IV; Ref. 4). Current therapy for advanced disease consists of staging laparotomy and cytoreductive surgery (5), followed by chemotherapy with platinum-containing complexes and Taxol (2, 6). Despite initially encouraging response rates of up to 70%, most women develop recurrent disease (7). Thus, given the high response rate and the low 5-year survival rate, cisplatin resistance is a critically important factor that limits the clinical utility of this agent.

In view of the central problem of cisplatin resistance, efforts have focused on the development of alternative platinum-based analogues that can be more effective against resistant disease. The compound DACH-acetato-Pt,[3] which bears axial acetate ligands (Fig. 1), represents a novel class of plati-

Received 5/27/98; revised 12/9/98; accepted 12/10/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by American Cancer Society Grant DHP-134 and the Sandra S. Davis Ovarian Cancer Research Fund (to Z. H. S.) and by a grant from the Office of Education and the University Cancer Foundation of the University of Texas M. D. Anderson Cancer Center (to G. S. H.). This work was presented in preliminary form at the 87th Annual Meeting of the American Association for Cancer Research, held April 20–24, 1996, in Washington, DC.

[2] To whom requests for reprints should be addressed, at Department of Clinical Investigation, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-7746; Fax: (713) 792-6759; E-mail: zsiddik@mdanderson.org.

[3] The abbreviations used are: DACH-acetato-Pt, (*1R,2R*-diaminocyclohexane)(*trans*-diacetato)(dichloro)-platinum(IV); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HPV 16, human papillomavirus type 16.

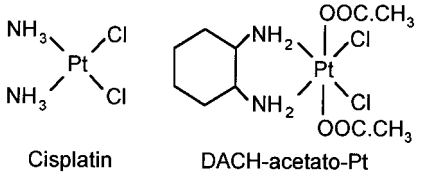

Fig. 1 Structure of platinum complexes.

num-based antitumor agents. The ability of DACH-acetato-Pt to circumvent resistance acquired through exposure to cisplatin is a particularly important observation of our laboratory (8), and mechanistic studies have been in progress as part of its preclinical development for the anticipated clinical trials.

Although the molecular basis of drug sensitivity and resistance is complex, the p53 tumor suppressor protein appears to play a major role in modulating cellular response to therapeutic agents. However, the impact of *p53* gene status on chemo- and radiosensitivity varies between different tumor types. This is a function of *(a)* the integrity of the cellular transduction pathways of the cell activated in response to injury, *(b)* the specific type of cellular damage produced by the drug, and *(c)* the inherent cell-specific responses of the particular cell (9). In cells that are programmed for apoptosis (especially hematopoietic and lymphoid cells), loss of p53 function decreases their sensitivity to a wide variety of DNA-damaging agents (10–17). This, however, is not observed in other cell types (18, 19). Presence of wild-type p53, on the other hand, does not necessarily ensure a chemosensitive phenotype, and a recent clinical study has underscored this very convincingly: Righetti *et al*. (20) reported that, among the group of chemoresistant ovarian tumors, 37% had wild-type *p53* and 63% had mutant *p53*. It is also noteworthy that, contrary to expectations, inactivation of wild-type p53 in MCF-7 breast carcinoma cells and in normal human foreskin fibroblasts enhanced sensitivity of these specific cells to cisplatin and other selected chemotherapeutic agents (21, 22).

This study was undertaken to assess the extent of cisplatin resistance in established human ovarian cancer lines with wild-type, mutant, or null *p53* status and to examine whether *p53* status modulates the ability of DACH-acetato-Pt to circumvent this resistance. We report here that, against cisplatin-resistant tumor cells with wild-type *p53*, DACH-acetato-Pt was significantly more effective and, unlike cisplatin, caused these cells to arrest in G_1 in a p53-dependent manner.

MATERIALS AND METHODS

Chemicals. Cisplatin was synthesized by us using a standard procedure reported previously (23). We have reported the synthesis and chemical characterization of DACH-acetato-Pt separately (24). Cisplatin and DACH-acetato-Pt were dissolved in normal saline and water, respectively, then sterilized through 0.22-μm disc filters. The concentration of each drug was confirmed by its platinum metal content, as determined by flameless atomic absorption spectroscopy (25), and then the drugs were diluted to 2 mM stock solutions. MTT was purchased from Sigma Chemical Co. (St. Louis, MO), and FCS was obtained from BioWhittaker, Inc. (Walkersville, MD).

Table 1 *p53* status of ovarian cancer cell lines

Cell line	*p53* gene status	Ref.
OVCA-420	Wild-type	31
OVCA-429	Wild-type	31
OVCA-433	Wild-type	31
OVCAR-10	Wild-type	—[a]
HEY	Wild-type	32
OVCA-432	Mutant	31
OVCAR-3	Mutant	33
OCC1	Mutant	32
SKOV-3	Null	33

[a] S. W. Johnson, personal communication.

Cell Lines. The nine ovarian carcinoma cell lines used in this study were established from biopsies taken from patients who had failed cisplatin- and/or alkylating agent-based chemotherapy and were classified as cisplatin resistant (26–30). The *p53* statuses of these cell lines have been documented (31–33) and are indicated in Table 1. Cells were grown as monolayers in 5% CO_2 and 95% humidified air at 37°C. The OVCA-420, -429, -432, and -433 cell lines were maintained in Eagle's MEM with Earle's salts containing 10% heat-inactivated FCS, 1 mM L-glutamine, 1 mM sodium pyruvate, 1 mM nonessential amino acids, and antibiotics (100 μg/ml streptomycin and 100 units/ml penicillin). The SKOV-3 cell line was grown in McCoy's 5a medium, 15% heat-inactivated FCS, and 2 mM L-glutamine. The HEY, OCC1, OVCAR-3, and OVCAR-10 cell lines were maintained in RPMI 1640 containing 10% heat-inactivated FCS, and 2 mM L-glutamine. The McCoy's 5a medium and RPMI 1640 were both supplemented with an antibiotic cocktail (100 μg/ml streptomycin, 100 units/ml penicillin, and 100 μg/ml neomycin).

HPV 16 E6 Transfection Studies. The HPV 16 E6 gene was cloned into a pCMV plasmid that also contains a neomycin gene (34). This plasmid and the pCMV-neo control vector were obtained from Dr. K. R. Cho (Johns Hopkins University School of Medicine, Baltimore, MD). For transfections, 1×10^6 OVCA-429 cells were seeded in a 100-mm tissue culture dish and incubated until 50–80% confluent. The HPV 16 E6 gene (34) or the pCMV-neo control vector were introduced into cells using Lipofectamine (Life Technologies, Inc.). Approximately 14 days later, G418-resistant colonies were selected and expanded.

The clones were examined for their stable growth characteristics and/or expression of *p53*. The HPV 16 E6 protein facilitates the degradation of the p53 protein via the ubiquitin-dependent proteolytic pathway (35). Therefore, a reduced level of p53 protein by Western analysis was used as an indication of clones with positive HPV 16 E6-mediated effects. Three such E6 clones were selected, along with two control transfectants, for experimental investigations. These selected clones were maintained in complete Eagle's MEM supplemented with 800 μg/ml G418. The clones were cultured in G418-free medium for one passage prior to undertaking experiments.

Cytotoxic Evaluations. Cytotoxicity was determined by a modified MTT assay (36), which has been validated against

Table 2 IC_{50} of cisplatin and DACH-acetato-Pt in wild-type, mutant, and null *p53* ovarian cancer cell lines following continuous or 2-h drug exposures

	Continuous drug exposure			2-h drug exposure		
Cell line	Cisplatin	DACH-acetato-Pt	Cisplatin/DACH-acetato-Pt ratio	Cisplatin	DACH-acetato-Pt	Cisplatin/DACH-acetato-Pt ratio
Wild-type *p53*						
OVCA-420	2.93 ± 0.18[a]	1.49 ± 0.24	1.97	21.2 ± 1.73	8.39 ± 0.81	2.53
OVCA-429	4.10 ± 0.63	0.79 ± 0.10	5.19	24.8 ± 1.67	5.61 ± 0.75	4.42
OVCA-433	9.90 ± 2.86	1.07 ± 0.33	9.25	103.3 ± 9.70	9.83 ± 1.39	10.5
OVCAR-10	8.90 ± 1.77	0.17 ± 0.01	52.4	109.5 ± 12.2	6.32 ± 0.53	17.3
HEY	2.78 ± 0.23	0.45 ± 0.05	6.18	29.9 ± 2.14	8.08 ± 0.98	3.70
Mutant or null *p53*						
OVCA-432	2.03 ± 0.25	11.3 ± 2.08	0.18	16.1 ± 1.91	73.2 ± 7.33	0.22
OVCAR-3	1.22 ± 0.33	2.65 ± 0.76	0.46	6.64 ± 0.98	23.5 ± 2.83	0.28
OCC1	3.30 ± 0.92	9.85 ± 3.65	0.34	19.3 ± 2.21	108.1 ± 10.3	0.18
SKOV-3	1.32 ± 0.17	3.18 ± 0.57	0.42	8.87 ± 1.10	44.2 ± 3.81	0.20

[a] Mean ± SE; $n = 3$–5.

the clonogenic assay in the evaluation of new platinum compounds (37). Cells in exponential growth were trypsinized, counted using a hemocytometer, and then diluted to appropriate concentrations. Aliquots (100 μl) of cell suspensions were added to each well of a 96-well microtiter plate. The stock cisplatin or DACH-acetato-Pt solution was serially diluted with complete medium immediately before use. Each diluted solution (100 μl) was added to wells in triplicate, and the cells were incubated at a 37°C in a 5% CO_2 humidified incubator. After 3 or 5 days, when untreated control cells were in logarithmic growth, 50-μl aliquots of an MTT solution (3 mg/ml) were added to the wells, and the microtiter plate was incubated for a further 3 h. The medium was then removed from wells and replaced with 50 μl of 100% DMSO to dissolve MTT formazan crystals. Plates were then agitated on a shaker for 5–10 min, and absorbances were measured at 570 nm with a multiwell scanning spectrophotometer (Dynatech, Chantilly, VA; or Molecular Devices, Sunnyvale, CA).

For cytotoxic evaluations using 2-h drug exposures, 100-μl aliquots of cell suspensions were placed in the 96-well microtiter plate. Following a 2-day attachment period in a 37°C-5% CO_2 humidified incubator, 100-μl aliquots of either complete medium or cisplatin or DACH-acetato-Pt solution in medium were added to wells in triplicate. After 2 h, the cells were washed free of the drug and then incubated in drug-free medium for a further 3 or 5 days. The MTT assay was then performed as described above.

The IC_{50}s, defined as the drug concentration (μM) inhibiting cell growth by 50% compared to control cells, were determined from a plot of log concentration *versus* A_{570} readings (as a percentage of control).

Cell Cycle Studies. Attached cells in an exponential growth phase in 100-mm tissue culture dishes were exposed for 2 h to cisplatin or DACH-acetato-Pt, as described above. The cells were washed and reincubated in drug-free medium. At appropriate time intervals, cells were collected, washed twice with ice-cold PBS, counted, and diluted with PBS to give a concentration of 1×10^6 cells/ml. Paraformaldehyde in PBS was added dropwise to the cell suspension to give a 1% final concentration. The cell suspension was then incubated for 15 min on ice. After this time, the cells were washed with ice-cold PBS and resuspended in ice-cold 70% ethanol at a concentration of 1×10^6 cells/ml. The suspension was stored at −20°C until completion of each set of experiments. Before analysis, the cell suspension was thawed, washed once with ice-cold PBS, resuspended in a solution of propidium iodide (10 μg/ml) in PBS containing 0.5% Tween 20 and 500 units/ml of RNase A (Sigma) and incubated at room temperature for 30 min and then at 4°C overnight. Cell cycle kinetics were determined on a Becton Dickinson flow cytometer.

Western Analysis. Cells were exposed for 2 h to cisplatin or DACH-acetato-Pt as described above, washed, and incubated at 37°C in drug-free medium for 24 h. The cells were then washed with PBS and lysed for 10 min on ice with 1 ml of lysis buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 1% NP40, 0.1% SDS, 0.5% sodium deoxycholate, 100 μg/ml phenylmethylsulfonyl fluoride, and 1 μg/ml aprotinin]. The lysates were collected by microcentrifugation at 4°C, and the protein level was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL). Forty μg of total cell protein were electrophoresed on a 10% SDS-polyacrylamide gel, blotted overnight in TBS-20 buffer [10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.05% Tween 20] containing 3% nonfat milk powder and 0.2% BSA. The membranes were then probed for 2 h with either the DO-1 (for mutant and wild-type p53; Oncogene Science, Cambridge MA) or the sdi1 (for $p21^{Waf1/Cip1}$; PharMingen, San Diego, CA) antibody. The antibody reaction was visualized by chemiluminescence using a sheep antimouse horseradish peroxidase as a second antibody (Amersham, Arlington Heights, IL) and quantified by laser densitometry. The membranes were then stripped of antibody with a stripping buffer [2% SDS, 62.5 mM Tris-HCl (pH 6.8), and 100 mM 2-mercaptoethanol] at 50°C for 30 min, washed with PBS-Tween 20, and then reprobed with a β-actin antibody (Sigma) to ensure equal loading of samples.

Statistical Analysis. Differences between groups were evaluated by Student's *t* test and were considered significant for values of $P < 0.05$.

Table 3 IC_{50} of cisplatin and DACH-acetato-Pt in control clone PVC1 and HPV E6 clone E6B2 from OVCA-429 cells and corresponding resistance factors for E6B2[a]

Parameter	Continuous drug exposure		2-h drug exposure	
	Cisplatin	DACH-acetato-Pt	Cisplatin	DACH-acetato-Pt
IC_{50}, PVC1	6.41 ± 0.83[b]	1.04 ± 0.17	22.2 ± 2.06	5.26 ± 0.50
IC_{50}, E6B2	6.73 ± 1.54	2.55 ± 0.58[c]	23.6 ± 2.85	26.3 ± 2.55[c]
Resistance factor	1.03 ± 0.19	3.25 ± 1.11[d]	1.06 ± 0.09	4.99 ± 0.12[d]

[a] Resistance factor of E6B2 = (IC_{50} *vs.* E6B2)/(IC_{50} *vs.* PVC1).
[b] Mean ± SE; $n = 3–7$.
[c] Significantly different from PVC1 ($P < 0.05$).
[d] Significantly different from 1.00 ($P < 0.05$).

RESULTS

Cytotoxicity of Cisplatin and DACH-acetato-Pt. Cytotoxic evaluations were conducted in nine cisplatin-resistant ovarian cancer cell lines of differing *p53* status (Table 1), and the results are shown in Table 2. Using the continuous drug exposure protocol, we found the IC_{50} for cisplatin in the wild-type *p53* cell lines to be in the range of 2.78–9.9 μM, with a median value of 4.1 μM. The IC_{50} for the mutant/null *p53* cell lines, on the other hand, ranged from 1.22 to 3.3 μM, with a median of ~1.32–2.03 μM. Thus, cisplatin was more effective against mutant/null *p53* cell lines than against the wild-type *p53* lines. With DACH-acetato-Pt, the median IC_{50} in wild-type *p53* cell lines was 0.79 μM (range, 0.17–1.49 μM). The median value increased to 3.18–9.85 μM (range, 2.65–11.3 μM) in mutant/null *p53* cell lines. It is apparent, therefore, that wild-type *p53* cell lines were more sensitive to DACH-acetato-Pt than mutant/null *p53* cell lines. This relationship is in sharp contrast to that seen with cisplatin. The differential cytotoxic profile of cisplatin and DACH-acetato-Pt is readily apparent by examining the ratio of IC_{50} for the two agents (Table 2). This ratio was >1 in wild-type *p53* cell lines and <1 in the mutant/null *p53* models. The difference between the ratios was statistically significant ($P < 0.05$), and this further demonstrates the superiority of DACH-acetato-Pt in wild-type *p53* cell lines. Similar results were obtained when drug exposure to cells was limited to 2 h (Table 2). However, the IC_{50}s for cisplatin and DACH-acetato-Pt following pulse exposures were 8.1-fold (SE, 0.83) and 13.0-fold (SE, 3.3) greater, respectively, than those following continuous drug exposures. These increases in IC_{50} for the 2-h exposure were expected and were similar to the 9–10-fold increases reported in human ovarian tumor models for cisplatin (29) and DACH-Pt(IV) analogues (37).

To confirm the role of wild-type *p53* in mediating the superior cytotoxic activity of DACH-acetato-Pt, we established stable transfectants of the OVCA-429 cell line with the HPV 16 E6 plasmid to inactivate *p53* function. The results with the control PVC1 and the test E6B2 clones indicate that loss of p53 function significantly increased resistance to DACH-acetato-Pt by ~3–5-fold ($P < 0.05$; Table 3). In contrast, no changes in resistance to cisplatin were noted.

Cell Cycle Kinetics following Exposure to Cisplatin and DACH-acetato-Pt. To gain insights into the mechanism of action of DACH-acetato-Pt, cell cycle studies were performed with two tumor models. With both wild-type *p53* OVCA-433 and mutant *p53* OVCA-432 cell lines, IC_{50} concentrations of cisplatin arrested cells in G_2 (Fig. 2, *A* and *C*): by 36–48 h, most of the cells were in G_2-M. In contrast, equitoxic concentrations of DACH-acetato-Pt demonstrated an interesting *p53*-dependent differential effect on cell cycle kinetics. In the OVCA-433 model with wild-type *p53*, the analogue arrested cells in G_1, with a concomitant decrease in the S-phase population (Fig. 2*B*). The kinetic profile returned to normal by 48 h. No accumulation in G_1 was observed in mutant *p53* OVCA-432 cells exposed to DACH-acetato-Pt. Instead, the analogue behaved like cisplatin and maximally arrested cells in G_2-M by 48 h (Fig. 2*D*). In these resistant tumor cell lines, associations between G_2-M arrest and low cytotoxicity of the analogue and between G_1 arrest and high activity are apparent.

Whether the G_1 arrest in wild-type *p53* cells by DACH-acetato-Pt was mediated in a *p53*-dependent manner was examined in wild-type *p53* OVCA-429 parental cells and its control and E6 transfectant clones. In parental cells, cisplatin induced the characteristic G_2-M arrest by 24–48 h and a concomitant decrease in the G_1 population (Fig. 3*A*). This was similar to the effect seen in OVCA-432 and -433 cells (Fig. 2, *A* and *C*). DACH-acetato-Pt caused OVCA-429 cells to arrest in G_1 and a decrease in S-phase (Fig. 3*B*), which is consistent with observations made with OVCA-433 cells (Fig. 2*B*). The effect of equitoxic concentrations of cisplatin and DACH-acetato-Pt on cell cycle distribution of control transfectant PVA1 and PVC1 clonal cells were similar to those observed in parental cells (Fig. 4). However, the characteristic G_1 arrest produced by DACH-acetato-Pt was abrogated by the HPV 16 E6 plasmid in the three E6 clones (E6B2, E6B4, and E6C6; Fig. 4*A*). Indeed, there were no gross differences between cisplatin and the analogue with regard to distribution of E6 transfectant cells in G_1, S, or G_2-M (Fig. 4). The results indicate that the presence of wild-type *p53* is important for DACH-acetato-Pt to mediate cellular effects with greater potency.

p53 and $p21^{Waf1/Cip1}$ Induction by Cisplatin and DACH-acetato-Pt. To investigate why cisplatin-resistant ovarian cancer cells with wild-type *p53* were sensitive to DACH-acetato-Pt, we examined p53 induction by the platinum agents in selected models. Increases in $p21^{Waf1/Cip1}$ were also determined as an indication of the transactivation potential of the *p53*-dependent *waf1/cip1* gene and to correlate with the G_1 arrest induced by DACH-acetato-Pt. The mutant p53 OVCA-432 cells, which overexpress *p53*, demonstrated no detectable changes in p53 protein levels 24 h after a 2-h exposure to equitoxic concentrations of cisplatin (up to 80 μM) or DACH-

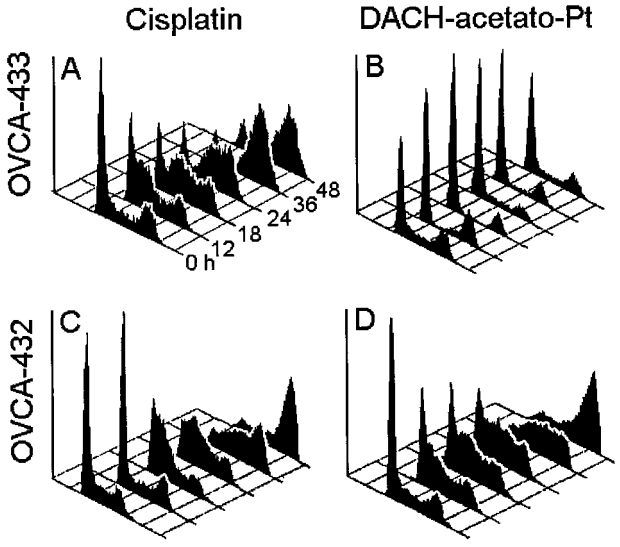

Fig. 2 Cell cycle analysis of the wild-type *p53* OVCA-433 and the mutant *p53* OVCA-432 cell lines exposed to cisplatin or DACH-acetato-Pt. Attached cells in an exponential growth phase were exposed for 2 h to cisplatin or DACH-acetato-Pt. The cells were then washed and reincubated in drug-free medium. Cells were harvested at 12, 18, 24, 36, and 48 h and analyzed by flow cytometry. *A*, OVCA-433, 100 μM cisplatin. *B*, OVCA-433, 10 μM DACH-acetato-Pt. *C*, OVCA-432, 15 μM cisplatin. *D*, OVCA-432, 70 μM DACH-acetato-Pt.

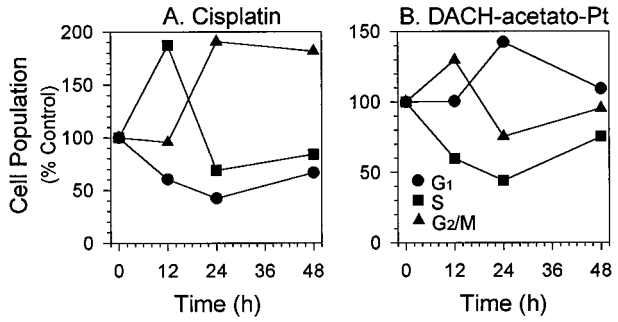

Fig. 3 Cell cycle analysis of the wild-type *p53* OVCA-429 cell line exposed to cisplatin or DACH-acetato-Pt. Attached cells in an exponential growth phase were exposed for 2 h to drug vehicle (control), 25 μM cisplatin, or 5 μM DACH-acetato-Pt. The cells were then washed and reincubated in drug-free medium. Cells were harvested at 12, 24, and 48 h and analyzed by flow cytometry. *A*, cisplatin. *B*, DACH-acetato-Pt.

acetato-Pt (up to 400 μM; data not shown). Levels of $p21^{Waf1/Cip1}$ were undetectable in these cells before or after drug treatment. The wild-type *p53* OVCA-429 cell line also expressed detectable basal levels of p53 (Fig. 5), but these were substantially lower than those observed in OVCA-432 cells. With cisplatin, a significant induction (≥20%) of p53, with coordinate increases in $p21^{Waf1/Cip1}$, was only observed in OVCA-429 cells at the high concentration of 125 μM (~5× IC_{50}). In comparison, DACH-acetato-Pt increased levels of both p53 and $p21^{Waf1/Cip1}$ at the lower drug concentration of 5 μM (~IC_{50}). Interestingly, the increases in $p21^{Waf1/Cip1}$ were only observed in parallel with p53 induction, which suggests that the induced p53 was functional with regard to its ability to transactivate the *waf1/cip1* gene. Similar differential effects of cisplatin and the analogue on p53 induction were also seen in wild-type *p53* OVCA-433 tumor cells (data not shown). Thus, the greater cytotoxicity of DACH-acetato-Pt against cisplatin-resistant ovarian tumor cells with wild-type status may relate to its ability to induce *p53* and $p21^{Waf1/Cip1}$ expression.

To explain the reduction in cytotoxicity of DACH-acetato-Pt by E6, levels of p53 and $p21^{Waf1/Cip1}$ were also examined in the control PVC1 and E6-transfectant E6B2 clones of OVCA-429 cells. Compared to parental OVCA-429 cells, untreated PVC1 cells expressed ~1.5-fold greater levels of p53 and 2.4-fold greater levels of $p21^{Waf1/Cip1}$ (Fig. 6). As with parental cells, cisplatin increased p53 by ~30% in PVC1 cells at the high 125 μM drug concentration only. Surprisingly, there was no corresponding increase in $p21^{Waf1/Cip1}$, which may be due to selective desensitization of transactivation mechanisms by the increased basal expression of *p53* in the PVC1 clone. DACH-acetato-Pt, on the other hand, induced both p53 and $p21^{Waf1/Cip1}$ in PVC1 cells at a lower drug concentration (Fig. 6), and this was consistent with results obtained in parental cells (Fig. 5). However, increases in p53 and $p21^{Waf1/Cip1}$ were lower, and this again may be related to higher basal levels of these proteins in the control transfectant. In contrast, the E6 plasmid reduced basal levels of p53 in E6B2 clone by ~30% compared to parental OVCA-429 cells but had no effect on $p21^{Waf1/Cip1}$ levels (Fig. 6). Both cisplatin and DACH-acetato-Pt failed to induce p53 or $p21^{Waf1/Cip1}$ in this clone. The results indicate that E6 abrogates drug-induced up-regulation of *p53* and $p21^{Waf1/Cip1}$, and this is consistent with cytotoxicity and cell cycle data obtained with the analogue from the E6B2 transfectant.

DISCUSSION

The initial reports on the activity of cisplatin were made three decades ago by Rosenberg *et al.* (38, 39). Since that time, analogue development has been a major priority and has centered on both improving the toxicity profile of cisplatin and circumventing cisplatin resistance (40). Our efforts have resulted in the identification of DACH-acetato-Pt as an analogue with clinical potential against cisplatin-resistant disease (8). This study reveals that this analogue is selectively active in cisplatin-resistant ovarian tumor cells harboring wild-type *p53*.

The presence of wild-type *p53* in tumor cells generally correlates with a good clinical response to drug therapy (41). The cytotoxic effect is usually preceded by drug-mediated cellular accumulation of wild-type p53. This induction can activate p53, which can then transactivate a number of genes to regulate cell cycle and apoptosis. The *p53*-dependent transactivation of $p21^{Waf1/Cip1}$ gene, for instance, appears to be essential in arresting cells in G_1 of the cell cycle. The *p53*-dependent expression of the *bax* gene, on the other hand, triggers apoptosis as a facile cytotoxic response to chemotherapeutic agents. Not surprisingly, loss of apoptotic functions appears to be a major cause of resistance to cytotoxic drugs (42–44). Consistent with this understanding is our observation here that cisplatin-resistant ovarian cells harboring wild-type *p53* were resistant to induction of p53 by cisplatin, whereas the greater cytotoxicity of DACH-

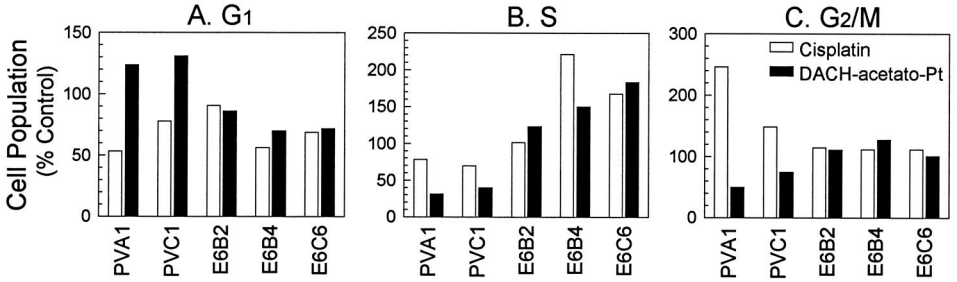

Fig. 4 The effects of cisplatin or DACH-acetato-Pt on cell cycle kinetics of three HPV 16 E6 clones (*E6B2*, *E6B4*, and *E6C6*) and two control vector clones (*PVA1* and *PVC1*) derived from the wild-type *p53* OVCA-429 cell line. Attached cells in an exponential-growth phase were exposed for 2 h to drug vehicle (control), 25 μM cisplatin, or 5 (control clones) or 25 (E6 clones) μM DACH-acetato-Pt. The cells were then washed and reincubated in drug-free medium. Cells were harvested at 24 h and analyzed by flow cytometry. *A*, G_1. *B*, S phase. *C*, G_2-M.

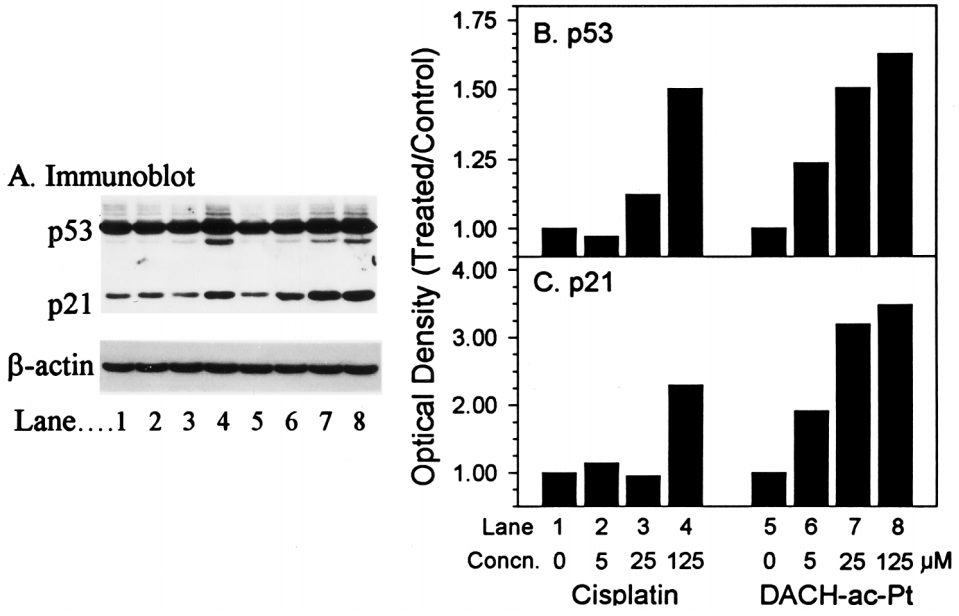

Fig. 5 Induction of p53 and $p21^{Waf1/Cip1}$ in the wild-type *p53* OVCA-429 cell line exposed to cisplatin or DACH-acetato-Pt. Cells in exponential growth phase were exposed to 0–125 μM of cisplatin or DACH-acetato-Pt (*DACH-ac-Pt*) for 2 h, washed and then incubated in drug-free medium. Cells were harvested 24 h later, and protein was extracted and examined for p53 and $p21^{Waf1/Cip1}$ levels by Western blotting. *A*, immunoblot analysis of p53 and $p21^{Waf1/Cip1}$. *B*, levels of p53 (relative to control in *Lane 1* or *5*) estimated from immunoblots by laser densitometry. *C*, levels of $p21^{Waf1/Cip1}$ (relative to control in *Lane 1* or *5*) estimated from immunoblots by laser densitometry.

acetato-Pt in these cells was associated with significant induction of p53 and $p21^{Waf1/Cip1}$. Furthermore, the analogue was considerably less cytotoxic against cisplatin-resistant cells with mutant *p53*. The demonstration of a loss in the activity of DACH-acetato-Pt in the wild-type *p53* OVCA-429 cell line expressing HPV 16 E6, which inactivates the p53 protein via a ubiquitin-dependent pathway (34, 35, 45), confirms the significant role of wild-type p53 and its induction in the cytotoxicity of the analogue. In contrast, the lack of change in the cytotoxicity of cisplatin in the E6 transfectant clone provides credence to the idea that p53 does not participate in mediating the cytotoxic effect of cisplatin in the OVCA-429 model.

The differential molecular and associated cytotoxic effects of cisplatin and DACH-acetato-Pt in resistant wild-type *p53* ovarian tumor cell lines are novel observations among platinum antitumor agents. The explanation for the results is not currently known but may be reconciled by considering that the two platinum drugs activate independent signal transduction pathways in response to DNA damage. It is likely that the normal pathway used by cisplatin in resistant wild-type *p53* ovarian cells is down-regulated, which prevents the characteristic induction of p53 and $p21^{Waf1/Cip1}$ proteins in response to DNA damage by clinically relevant concentrations of cisplatin. DACH-acetato-Pt, on the other hand, may use an alternative pathway that appears to be intact and fully capable of regulating p53 levels in response to DNA damage. The existence of alternative pathways for p53 induction has been implicated previously from studies with ataxia-telagiectasia cells. Artuso *et al.* (46) and Zhang *et al.* (47), for instance, reported that p53 induction was poor following treatment with ionizing radiation but was comparable to that in normal cells treated with methylmethane sulfonate, cisplatin, or UV light. Similarly, we have found in a separate study that the cisplatin-resistant ovarian 2780CP model lacked the ability to accumulate p53 in response to cisplatin, but induction of p53 and p53-mediated functions was normal when cells were exposed to X-rays (48). On the basis of these results, it may be reasonable to speculate that methylmethane sulfonate, cisplatin, and UV light activate sig-

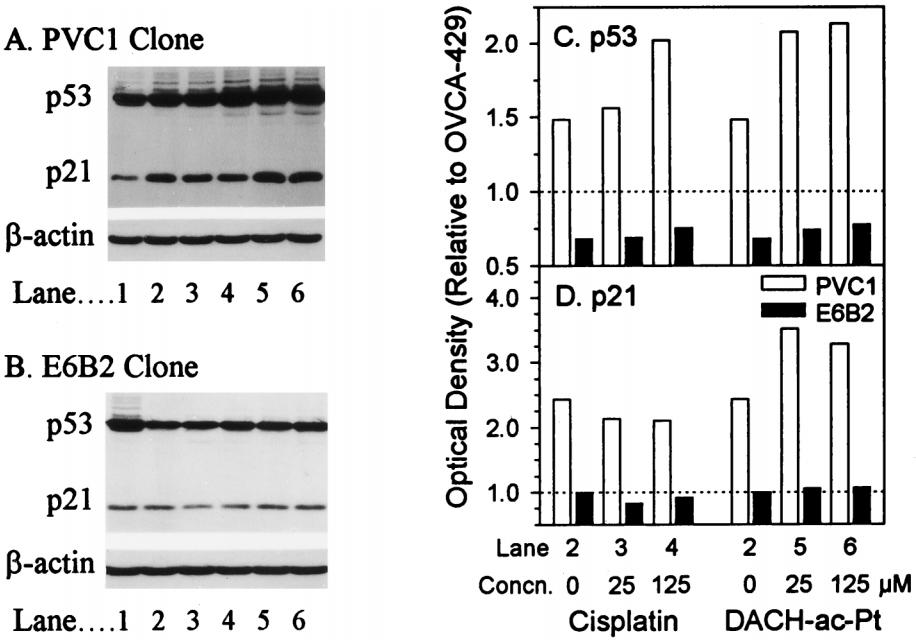

Fig. 6 Induction of p53 and $p21^{Waf1/Cip1}$ by cisplatin or DACH-acetato-Pt in the HPV 16 E6 clone E6B2 and the control vector PVC1 clone derived from the wild-type *p53* OVCA-429 cell line. Cells in exponential growth phase were exposed to 0, 25, or 125 μM cisplatin or DACH-acetato-Pt (*DACH-ac-Pt*) for 2 h, washed, and then incubated in drug-free medium. Cells were harvested 24 h later, and protein was extracted and examined for p53 and $p21^{Waf1/Cip1}$ levels by Western blotting. *A*, immunoblot analysis of p53 and $p21^{Waf1/Cip1}$ for the PVC1 clone. *B*, immunoblot analysis of p53 and $p21^{Waf1/Cip1}$ for the E6B2 clone. *C*, levels of p53 relative to those in parental OVCA-429 cells (relative to *Lanes 1*) estimated from immunoblots by laser densitometry. *D*, levels of $p21^{Waf1/Cip1}$ (relative to *Lanes 1*) estimated from immunoblots by laser densitometry.

naling pathways that are distinct from those activated by DACH-acetato-Pt and ionizing radiation.

Apart from the differential effect of cisplatin and DACH-acetato-Pt on p53 and $p21^{Waf1/Cip1}$ levels, a second observation of interest noted in this study was the relative abilities of the two agents to induce G_1 arrest. DACH-acetato-Pt demonstrated an ability to arrest wild-type *p53* cells in G_1, but this was not apparent with cisplatin. It is well acknowledged that arrest in the G_1 phase of the cell cycle after DNA damage requires participation of wild-type p53 protein (9, 43, 49). Furthermore, transcriptional activation of $p21^{Waf1/Cip1}$ is necessary for p53-mediated G_1 arrest by a number of agents, including γ-rays and Adriamycin (50). Thus, the differential effect of the two platinum agents at G_1 of the cell cycle is consistent with their relative abilities to increase intracellular levels of $p21^{Waf1/Cip1}$. However, it needs to be stressed that the cisplatin-mediated effect on the cell cycle is generally characterized by a predominant G_2-M arrest (51–53), even in cells in which cisplatin induces wild-type p53 and $p21^{Waf1/Cip1}$ (54). Interestingly, the analogue induced G_2 arrest in cells with mutant *p53*, an observation that is consistent with literature reports on cells that lack functional p53. We confirmed this in this study using transfectant clones, in which p53 inactivation by HPV 16 E6 expression abrogated the prominent G_1 accumulation and produced G_2-M arrest instead. It is not known at this stage if the G_1 arrest mediated by DACH-acetato-Pt is necessary for its cytotoxicity against wild-type *p53* cells. However, it is possible that the cytotoxic effects of the analogue may be distinct from its ability to induce G_1 arrest, as has been proposed from studies in other model systems (55, 56). Nevertheless, the ability of DACH-acetato-Pt to arrest cells in G_1 needs further investigation to better define its mechanism of action. It is feasible, by analogy with other G_1-arresting agents, that DACH-acetato-Pt may induce DNA strand breaks as opposed to the predominant formation of cross-links by cisplatin (57, 58).

Current therapy for effective management of ovarian carcinoma consists of cytoreductive surgery (5), followed by combination chemotherapy with platinum complexes and Taxol (6). Nevertheless, the 5-year survival rate of this disease is still dismal and has not changed significantly over the past 10 years (59). These statistics may change, however, as we begin to more fully comprehend the existence of multiple drug-specific signaling pathways and then use chemotherapeutic agents to activate specific pathways for affecting tumor cell kill. In this regard, the possibility that an alternative *p53* regulatory pathway participates in the activity of DACH-acetato-Pt may represent a potentially important development for clinical therapy of subset of platinum-resistant ovarian tumors with wild-type *p53* gene status. These results provide evidence for the novelty of DACH-acetato-Pt and endorse the development of this analogue for clinical trials.

ACKNOWLEDGMENTS

We thank Drs. Neely Atkinson, Jon Weiner, and Wei Zhang for helpful discussions and Ruth LaPushin, Long Nguyen, and Gerald Thai for expert technical assistance.

REFERENCES

1. Parker, S. L., Tong, T., Bolden, S., and Wingo, P. A. Cancer statistics, 1997. CA Cancer J. Clin., *47:* 5–27, 1997.

2. Thigpen, T., Vance, R., Khansur, T., and Malamud, F. The role of chemotherapy in the management of celomic epithelial carcinoma of the ovary. Cancer Invest., *15:* 277–287, 1997.

3. Steele, G. D., Jr., Osteen, R. T., Winchester, D. P., Murphy, G. P., and Menck, H. R. Clinical highlights from the National Cancer Data Base: 1994. CA Cancer J. Clin., *44:* 71–80, 1994.

4. Ozols, R. F. Ovarian cancer. Semin. Surg. Oncol., *6.* 328–338, 1990.

5. Benjamin, I., and Rubin, S. C. Initial surgical management of advanced epithelial ovarian cancer. Cancer Invest., *15:* 270–276, 1997.

6. McGuire, W. P., Hoskins, W. J., Brady, M. F., Kucera, P. R., Partridge, E. F., Look, K. Y., Clarke-Pearson, D. L., and Davidson, M.

Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N. Engl. J. Med., *334:* 1–6, 1996.

7. Runowicz, C. D. Advances in the screening and treatment of ovarian cancer. CA Cancer J. Clin., *42:* 327–349, 1992.

8. Kido, Y, Khokhar, A. R., Al-Baker, S., and Siddik, Z. H. Modulation of cytotoxicity and cellular pharmacology of 1,2-diaminocyclohexane platinum(IV) complexes mediated by axial and equatorial ligands. Cancer Res., *53:* 4567–4572, 1993.

9. O'Connor, P. M. Mammalian G_1 and G_2 phase checkpoints. *In:* M. Kastan (ed.), Cancer Surveys, Vol. 29: Checkpoint Controls and Cancer, pp. 151–182. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1997.

10. Fan, S., El-Deiry, W. S., Bae, I., Freeman, J., Jondle, D., Bhatia, K., Fornace, A. J., Jr., Magrath, I., Kohn, K. W., and O'Connor, P. M. *p53* gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents. Cancer Res., *54:* 5824–5830, 1994.

11. Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C. Hooper, M. L., and Wyllie, A. H. *p53* is required for radiation-induced apoptosis in mouse thymocytes. Nature (Lond.), *362:* 847–849, 1993.

12. Lowe, S. W., Ruley, H. E., Jacks, T., and Housman, D. E. *p53*-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell, *74:* 957–967, 1993.

13. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. Thymocyte apoptosis induced by *p53*-dependent and -independent pathways. Nature (Lond.), *362:* 849–852, 1993.

14. Radford, I. R., Murphy, J. M., Radley, J. M., and Ellis, S. L. Radiation response of mouse lymphoid and myeloid cell lines. Part II. Apoptotic death is shown by all lines examined. Int. J. Radiat. Biol., *65:* 217–227, 1994.

15. O'Connor, P. M., Jackman, J., Jondle, D., Bhatia, K., Magrath, I., and Kohn, K. W. Role of the *p53* tumor suppressor gene in cell cycle arrest and radiosensitivity of Burkitt's lymphoma cell lines. Cancer Res., *53:* 4776–4780, 1993.

16. Lee, J. M., and Bernstein, A. *p53* mutations increase resistance to ionizing radiation. Proc. Natl. Acad. Sci. USA, *90:* 5742–5746, 1993.

17. McIlwrath, A. J., Vasey, P. A., Ross, G. M., and Brown, R. Cell cycle arrests and radiosensitivity of human tumor cell lines: dependence on wild-type *p53* for radiosensitivity. Cancer Res., *54:* 3718–3722, 1994.

18. Slichenmyer, W. J., Nelson, W. G., Slebos, R. J., and Kastan, M. B. Loss of *p53*-associated G_1 checkpoint does not decrease cell survival following DNA damage. Cancer Res., *53:* 4164–4168, 1993.

19. Brachman, D. G., Beckett, M. Graves, D., Haraf, D., Vokes, E., and Weichselbaum, R. R. *p53* mutation does not correlate with radiosensitivity in 24 head and neck cancer cell lines. Cancer Res., *53:* 3667–3669, 1993.

20. Righetti, S. C., Torre, G. D., Pilotti, S., Menard, S., Ottone, F., Colnaghi, M. I., Pierotti, M. A., Lavarino, C., Cornarotti, M., Oriana, S., Bohm, S., Bresciani, G. L., Spatti, G., and Zunino, F. A comparative study of *p53* gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. Cancer Res., *56:* 689–693, 1996.

21. Fan, S., Smith, M. L., Rivet, D. J., Duba, D., Zhan, Q., Kohn, K. W., Fornace, A. J., Jr., and O'Connor, P. M. Disruption of *p53* sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. Cancer Res., *55:* 1649–1654, 1995.

22. Hawkins, D. S., Demers, G. W., and Galloway, D. A. Inactivation of *p53* enhances sensitivity to multiple chemotherapeutic agents. Cancer Res., *56:* 892–898, 1996.

23. Vollano, J. F., Al-Baker, S., Dabrowiak, J. C., and Schurig, J. E. Comparative anti-tumor studies on platinum(II) and platinum(IV) complexes containing 1,2-diaminocyclohexane. J. Med. Chem., *30:* 716–719, 1987.

24. Al-Baker, S., Siddik, Z. H., and Khokhar, A. R. Synthesis and characterization of new antitumor *trans-R,R-*, *trans-S,S-*, and *cis*-1,2-diaminocyclohexane platinum(IV) complexes. J. Coord. Chem., *31:* 109–116, 1994.

25. Siddik, Z. H., Boxall, F. E., and Harrap, K. R. Flameless atomic absorption spectrophotometric determination of platinum in tissues solubilized in hyamine hydroxide. Anal. Biochem., *163:* 21–26, 1987.

26. Bast, R. C., Jr., Feeney, M., Lazarus, H., Nadler, L. M., Colvin, R. B., and Knapp, R. C. Reactivity of a monoclonal antibody with human ovarian carcinomas. J. Clin. Invest., *68:* 1331–1337, 1981.

27. Xu, Y., Gaudette, D. C., Boynton, J. D., Frankel, A., Fang, X-J., Sharma, A., Hurteau, J., Casey, G., Goodbody, A., Mellors, A., Holub, B. J., and Mills, G. B. Characterization of an ovarian cancer activating factor in ascites from ovarian cancer patients. Clin. Cancer Res., *1:* 1223–1232, 1995.

28. Perez, R. P., O'Dwyer, P. J., Handel, L. M., Ozols, R. F., and Hamilton, T. C. Comparative cytotoxicity of CI-973, cisplatin, carboplatin and tetraplatin in human ovarian carcinoma cell lines. Int. J. Cancer, *48:* 265–269, 1991.

29. Kelland, L. R., and Abel, G. Comparative *in vitro* cytotoxicity of Taxol and taxotere against cisplatin-sensitive and -resistant human ovarian carcinoma cell lines. Cancer Chemother. Pharmacol., *30:* 444–450, 1992.

30. Buick, R. N., Pullano, R., and Trent, J. M. Comparative properties of five human ovarian adenocarcinoma cell lines. Cancer Res., *45:* 3668–3676, 1985.

31. Elbendary, A., Berchuck, A., Davis, P., Havrilesky, L., Bast, Jr., R. C., Iglehart, J. D., and Marks, J. R. Transforming growth factor β1 can induce *cip1/waf1* expression independent of the *p53* pathway in ovarian cancer cells. Cell Growth Differ., *5:* 1301–1307, 1994.

32. Wolf, J. K., Bazelle, L., Mills, G. B., Bast, R. C., Roth, J. A., and Gershenson, D. M. Growth inhibition of human ovarian cancer cells by transfection with adenovirus-mediated *p53* is independent of endogenous *p53* status. Proc. Am. Assoc. Cancer Res., *37:* 205, 1996.

33. Yaginuma, Y., and Westphal, H. Abnormal structure and expression of *p53* gene in human ovarian carcinoma cell lines. Cancer Res., *52:* 4196–4199, 1992.

34. Kessis, T. D., Slebos, R. J., Nelson W. G., Kastan, M. B., Plunkett, B. S., Han, S. M., Lorincz, A. T., Hedrick, L., and Cho, K. R. Human papillomavirus 16 E6 expression disrupts the *p53*-mediated cellular response to DNA damage. Proc. Natl. Acad. Sci. USA, *90:* 3988–3992, 1993.

35. Scheffner, M., Huibregtse, J. M., and Howley, P. M. Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of. p53. Proc. Natl. Acad. Sci. USA, *91:* 8797–8801, 1994.

36. Carmichael, J., Degraff, W., Gazdar, A., Minna, J., and Mitchell, J. Evaluation of a tetrazolium-based semi-automatic colorimetric assay: assessment of chemosensitivity testing. Cancer Res., *47:* 936–942, 1987.

37. Kido, Y, Khokhar, A. R., and Siddik Z. H. Differential cytotoxicity, uptake and DNA binding of tetraplatin and analogous isomers in sensitive and resistant cancer cells. Anti-Cancer Drugs, *4:* 251–258, 1993.

38. Rosenberg, B., Van Camp, L., and Krigas, T. Letter: inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. Nature (Lond.), *205:* 698–699, 1965.

39. Rosenberg, B., Van Camp, L., Trosco, J. E., and Mansour, V. H. Platinum compounds: a new class of potent antitumor agents. Nature (Lond.), *222:* 385–386, 1969.

40. Kelland, L. R. Prospects for improved cisplatin analogs. *In:* F. Sharp, T. Blackett, R. Leake, and J. Berek (eds.), Ovarian Cancer, Vol. 4, pp. 249–258. London: Chapman and Hall, 1996.

41. Ruley, H. E. *p53* and response to chemotherapy and radiotherapy. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Important Advances in Oncology, pp. 37–56. Philadelphia: Lippincott-Raven Publishers, 1996.

42. Fisher, D. E. Apoptosis in cancer therapy: crossing the threshold. Cell, *78:* 539–542, 1994.

43. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of *p53* protein in the cellular response to DNA damage. Cancer Res., *51:* 6304–6311, 1991.

44. Hartwell, L. H., and Kastan, M. B. Cell cycle control and cancer. Science (Washington DC), *266:* 1821–1828, 1994.

45. Scheffner, M., Werness, B. A., Huilbregtse, J. M., Levine, A. J., and Howley, P. M. The E6 oncoprotein encoded by the human papillomavirus types 16 and 18 promotes the degradation of p53. Cell, *63:* 1129–1136, 1990.

46. Artuso, M., Esteve, A., Bresil, H., Vuillaume, M., and Hall, J. The role of the ataxia-telangiectasia gene in the *p53*-, *waf1/cip1 (p21*)- and *GADD45*-mediated response to DNA damage produced by ionizing radiation. Oncogene, *11:* 1427–1435, 1995.

47. Zhang, N., Song, Q., Lu, H., and Lavin, M. F. Induction of *p53* and increased sensitivity to cisplatin in ataxia-telangiectasia cells. Oncogene, *13:* 655–659, 1996.

48. Siddik, Z. H., Mims, B., Lozano, G., and Thai, G. Independent pathways of *p53* induction by cisplatin and X-rays in a cisplatin-resistant ovarian tumor cell line. Cancer Res., *58:* 698–703, 1998.

49. Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V., and Kastan, M. B. Wild-type *p53* is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. USA, *89:* 7491–7495, 1992.

50. Waldman, T., Kinzler, K. W., and Vogelstein, B. p21 is necessary for the *p53*-mediated G_1 arrest in human cancer cells. Cancer Res., *55:* 5187–5190, 1995.

51. Ormerod, M. G., Orr, R. M., and Peacock, J. H. The role of apoptosis in cell killing by cisplatin: a flow cytometric study. Br. J. Cancer, *69:* 93–100, 1994.

52. Sorenson, C. M., Barry, M. A., and Eastman, A. Analysis of events associated with cell cycle arrest in G_2 phase and cell death induced by cisplatin. J. Natl. Cancer Inst. (Bethesda), *82:* 749–755, 1990.

53. Sorenson, C. M., and Eastman, A. Mechanism of *cis*-diamminedichloroplatinum(II)-induced cytotoxicity: role of G_2 arrest and DNA double strand breaks. Cancer Res., *48:* 4484–4488, 1988.

54. Demarcq, C., Bunch, R. T., Creswell, D., and Eastman, A. The role of cell cycle progression in cisplatin-induced apoptosis in Chinese hamster ovary cells. Cell Growth Differ., *5:* 983–993, 1994.

55. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J., and Leder, P. Mice lacking $p21^{CIP1/WAF1}$ undergo normal development, but are defective in G_1 checkpoint control. Cell, *82:* 675–684, 1995.

56. Attardi, L. D., Lowe, S. W., Brugarolas, J., and Jacks, T. Transcriptional activation by p53, but not induction of the *p21* gene, is essential for oncogene-mediated apoptosis. EMBO J., *15:* 3693–3701, 1996.

57. Pinto, A. L., and Lippard. S. J. Binding of the antitumor drug *cis*-diamminedichloro-platinum(II) (cisplatin) to DNA. Biochim. Biophys. Acta, *780:* 167–180, 1985.

58. Roberts, J. J., and Friedlos, F. Quantitative estimations of cisplatin-induced DNA interstrand cross-links and their repair in mammalian cells: relationship to toxicity. Pharmacol. Ther., *34:* 215–246, 1987.

59. Ries, L. A. G., Kosary, C. L., Hankey, B. F., Miller, B. A., and Edwards, B. K. (eds.). SEER Cancer Statistics Review, 1973–1993. Tables and Graphs. Bethesda: National Cancer Institute, 1996.

Clinical Cancer Research

Expression of *p53* in Cisplatin-resistant Ovarian Cancer Cell Lines: Modulation with the Novel Platinum Analogue (*1R*, *2R* -Diaminocyclohexane)(*trans*-diacetato)(dichloro)-platinum(IV)

George S. Hagopian, Gordon B. Mills, Abdul R. Khokhar, et al.

Clin Cancer Res 1999;5:655-663.