**E1A-mediated Paclitaxel Sensitization in HER-2/neu-overexpressing Ovarian Cancer SKOV3.ip1 through Apoptosis Involving the Caspase-3 Pathway**

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**ABSTRACT**

HER-2/neu-overexpressing breast cancer cells are more resistant to the chemotherapeutic agent paclitaxel (Taxol) than low-HER-2/neu-overexpressing breast cancer cells, and the adenoviral type 5 E1A can down-regulate HER-2/neu overexpression. Therefore, in this study, we asked (a) whether E1A might sensitize response to paclitaxel in human HER-2/neu-overexpressing ovarian cancer cells, and, if so, what is the mechanism responsible; and (b) whether this enhanced chemosensitivity would translate into a therapeutic effect in an ovarian cancer xenograft model. Consequently, we demonstrated that: (a) adenovirus type 5 E1A could enhance the sensitivity of paclitaxel in paclitaxel-resistant HER-2/neu-overexpressing human ovarian cancer cells in vitro by inducing apoptosis, (b) this induction was heavily dependent on activation of the caspase-3 pathway, and (c) nude mice bearing i.p. HER-2/neu-overexpressing human ovarian cancer cells and treated with both paclitaxel and E1A gene therapy survived significantly longer than did mice treated only with paclitaxel or E1A gene therapy. Thus, we concluded that the E1A gene enhanced both the in vitro and in vivo sensitivity of paclitaxel in paclitaxel-resistant HER-2/neu-overexpressing ovarian cancer SKOV3.ip1 cells. Because a Phase I clinical trial using E1A gene targeted to HER-2/neu down-regulation has recently been completed, the current study also provided a scientific basis to further develop a novel therapy that combines paclitaxel and E1A gene therapy and its testing in a Phase II trial.

**INTRODUCTION**

The HER-2/neu gene, which encodes a Mr 185,000 transmembrane growth factor receptor with tyrosine kinase activity (1–6), is amplified or overexpressed in approximately 20–30% of human ovarian cancers (7, 8). It is also well known that the long-term overall survival and disease-free survival rates for patients with HER-2/neu-overexpressing ovarian cancers are significantly lower than those for patients with cancers that do not overexpress HER-2/neu, thus indicating that HER-2/neu overexpression is a poor prognostic factor (7, 8). Additionally, it has recently been suggested that HER-2/neu overexpression may predict a poor response to chemotherapeutic agents (9, 10).

In a previously reported experimental model, enhanced expression of the HER-2/neu gene could increase tumorigenicity and experimental metastasis in mouse embryo fibroblasts and human cancer cells (11–15). HER-2/neu overexpression can also confer resistance to chemotherapeutic agents in non-small cell lung cancer and breast cancer cell lines (16–20). We have previously shown that HER-2/neu-overexpressing human breast cancer cell lines are highly resistant to the taxane chemotherapeutic agents paclitaxel and docetaxel (18–20). Collectively, all of the above data may explain, at least, in part, the poor clinical outcome for patients with HER-2/neu-overexpressing cancers.

Interestingly, HER-2/neu overexpression can be inhibited in both rodent cells and human breast and ovarian cancer cells by repressing the HER-2/neu promoter via the adenovirus type 5 E1A gene (21, 22), which encodes a well-known transcription factor (23). This inhibition abolishes the tumorigenicity and metastatic capability induced by the HER-2/neu oncogene (14, 24–28). Based on this preclinical finding, we recently completed a Phase I trial of E1A gene therapy in patients with advanced HER-2/neu-overexpressing breast and ovarian cancer cells.

On the basis of this information, it has been shown that E1A can sensitize HER-2/neu-overexpressing human breast cancer cells to the chemotherapeutic agent paclitaxel by down-regulating the expression of HER-2/neu in tissue culture (18). Other investigators have shown that E1A can enhance sensitivity to other chemotherapeutic agents in different types of cell lines, regardless of the HER-2/neu expression level, at least partially by induction of apoptosis through stabilization of p53 expression by E1A (29, 30). However, there is no preclinical animal data to show that E1A chemosensitization can translate to a therapeutic benefit, which would allow development of a novel therapeutic modality as a Phase II trial. Given these findings, we examined (a) whether E1A might sensitize response to pacli-
taxel in human HER-2/neu-overexpressing ovarian cancer cells and, if so, what mechanism is responsible, and (b) whether this enhanced chemosensitivity might be translated into a therapeutic effect in a human ovarian cancer xenograft animal model.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. All human ovarian cancer cell lines used were obtained from the American Type Culture Collection (Rockville, MD). SKOV3.ip1 cells (25) express only basal levels of HER-2/neu (31). All cell lines were grown in DMEM/F12 (Life Technologies, Inc., Grand Island, NY), supplemented with 10% fetal bovine serum and penicillin/streptomycin. A humidified incubator was set at 37°C; the air contained 5% CO₂.

Western Blot Analysis. Cells were washed three times with PBS and then lysed in lysis buffer (20 mM Na₂PO₄, (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% aprotinin, 1 mM phenylmethylsulfonyl fluoride, 100 mM NaF, and 2 mM SO₄) as described previously (32, 33). Protein content was determined against a standardized control using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). A total of 50 μg of protein was separated by 8–12% SDS-PAGE and transferred to nitrocellulose filter paper (Schleicher & Schuell, Inc., Keene, NH). Nonspecific binding on the nitrocellulose filter paper was minimized with a blocking buffer containing 5% nonfat dry milk and 0.1% v/v Tween 20 in PBS. The treated filter paper was then incubated, first with the primary antibody [anti-HER-2/neu antibody c-neu (Ab-3; Oncogene Science, Uniondale, NY) or anti-adenovirus type 5 EIA antibody m58 (PharMingen, San Diego, CA)] and then with the secondary antibody HRP-conjugated-goat antirabbit antibody (1:5,000 dilution; Jackson Immunoresearch, Westgrove, PA). Other primary and secondary antibodies used in the research reported here include anti-Bcl-2 antibody (1:500 dilution; PharMingen) with secondary antibody HRP-conjugated goat anti-Syrin hamster (1:1,000 dilution; Jackson Immunoresearch), anti-Bax antibody (1:500 dilution; Upstate Biotechnology, Lake Placid, NY) with secondary antibody HRP-conjugated goat antirabbit antibody (1:5,000 dilution), anti-PARP antibody (1:2,000 dilution; PharMingen) with secondary antibody HRP-conjugated goat antitumor mouse antibody (1:3,000 dilution; Jackson Immunoresearch), anti-caspase 3 antibody (1:1,000 dilution; PharMingen) with secondary antibody HRP-conjugated goat antirabbit antibody (1:5,000 dilution), anti-caspase-7 antibody (1:1,000 dilution; Transduction Laboratories, Lexington, KY) with secondary antibody HRP-conjugated goat antitumor mouse antibody (1:5,000 dilution), anti-Bad antibody (1:500 dilution; Transduction Laboratories) with secondary antibody HRP-conjugated goat antitumor mouse antibody (1:5,000 dilution), and anti-Bcl-XXL antibody (1:1,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) with secondary antibody HRP-conjugated goat antirabbit antibody (1:10,000 dilution).

Paclitaxel. A stock solution of paclitaxel (Bristol-Myers Squibb Co., Wallingford, CT) was stored at −80°C before use. At the time of use, paclitaxel was diluted in either DMEM/F12 or PBS.

DC-Chol Cationic Liposome. DC-Chol is a cationic derivative of cholesterol, 3-[N-(N',N'-dimethylaminoethoxy)-carbamoyl]cholesterol, and dioleoylphosphatidylethanolamine. A DNA/DC-Chol-dioleoylphosphatidylethanolamine cationic liposome complex was prepared by gently mixing 150 nmol of cationic liposome with 15 μg of plasmid.

Drug Sensitivity Assays. Cells were detached by trypsinization, seeded at 1.0–2.0 × 10⁵ cells/well in a 96-well microtiter plate overnight, and treated with different concentrations of paclitaxel in DMEM/F12 with 10% fetal bovine serum. The effects on cell growth were examined by MTT assay. Finally, 20 μL of MTT solution (5 mg/ml in PBS; Sigma Chemical Co.) were added to each well and incubated for 2 h at 37°C. The MTT-formazan formed by metabolically viable cells was dissolved in 100 μL of cell lysis buffer, and fluorescence was monitored by a microplate reader (Dynatech MR 5000 fluorescence; Dynatech Corp., Burlington, MA) at a wavelength of 570 nm (34).

Fluorescence-activated Cell Sorting Analysis. Analysis of apoptotic cells by flow cytometry was performed as described previously (35). Trypsinized cells were washed with PBS and then fixed with 70% ethanol. The fixed cells were kept at least overnight at −20°C. The cells were washed with PBS before analysis, and then the fluorochrome solution (50 μg/ml propidium iodide in 0.1% sodium citrate, 0.1% Triton X-100; Sigma Chemical Co.; plus RNase, 8–20 μg/ml) was added.

DNA Fragmentation Analysis. Cells were collected and resuspended in 300 μL of PBS to which 3 ml of extraction buffer [10 mM Tris (pH 8.0), 0.1 mM EDTA, 20 μg/mL RNase, and 0.5% SDS] were added. The cells were then incubated at 37°C for 1–2 h. After incubation, protease K was added to achieve a final concentration of 100 μg/ml. The solution was placed in a 50°C water bath for at least 3 h. DNA was extracted with an equal volume of phenol saturated with 0.5 mM Tris (pH 8.0), and then extracted again with a combination of phenol and chloroform. Precipitated DNA was analyzed on a 1.8% agarose gel.

Hoechst Staining and Caspase Inhibitor. For analysis of chromatin condensation, which is a sign of apoptosis, 2000 cells were added to each well of the 96-well plates. A cell-permeable caspase inhibitor [either Z-VAD-FMK (36) or Z-DEVD-FMK (Ref. 37; Enzyme System Products, Livermore, CA)] was then added to achieve a final concentration of 20 μM. After 1 h, the cells were exposed to paclitaxel (1 or 0.1 μM). Hoechst staining was performed 24 and 36 h later.

Hoechst 33342 (Sigma Chemical Co.) was diluted in distilled water (50 mg/ml), aliquoted, and stored as a stock solution at −20°C until use; for use, the stock solution was diluted with 8% (1:2500) formaldehyde (electron microscope grade) in PBS to a final concentration of 10–20 μg/ml. An equal volume of the diluted stock solution was added to each well in which cells had been exposed to either paclitaxel or caspase inhibitor or both. The cells were then incubated for

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The abbreviations used are: DMEM/F12, DMEM/Ham’s F-12; DC-Chol, 3α-[N-(N',N'-dimethylaminoethoxy)-carbamoyl] cholesterol, and dioleoylphosphatidyl ethanolamine; MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; HRP, horseradish peroxidase; PARP, poly(ADP)-ribose polymerase.
10 min at room temperature. Finally, each well was examined under a fluorescence microscope equipped with a DM455 filter for chromatin condensation; four individual wells were randomly selected and counted at high power. Three independent experiments were conducted.

**Animal Studies.** SKOV3.ip1 cells in log phase growth were trypsinized, washed twice with PBS, and centrifuged at 250 x g. Viable cells were counted. Two x 10⁶ cells in 0.5 ml of PBS were then injected i.p. under aseptic conditions into nude mice. After 5 days, tumor-bearing mice were randomly divided into four groups (five mice/group) for treatment with paclitaxel and/or E1A gene therapy: (a) group 1, untreated controls; (b) group 2, mice were given i.p. injections of E1A/DC-Chol complexes alone (15 µg of DNA and 200 nmol of liposome in a total volume of 200 µl/injection); (c) group 3, mice were given i.p. injections of paclitaxel alone (15 mg/kg in a total volume of 200 µl/injection) every 3 weeks; and (d) group 4, mice were given injections of E1A/DC-Chol complexes (15 µg of DNA and 200 nmol of liposome in a total volume of 200 µl/injection) and were given injections of paclitaxel 48 h later (15 mg/kg in a total volume of 200 µl/injection) every 3 weeks. Survival data were summarized and plotted using the method of Kaplan and Meier (38). The resulting survival curves were compared using log-rank tests.

**Fig. 1** Effects of paclitaxel on the growth of two human ovarian cancer cell lines exhibiting different levels of HER-2/neu and E1A expression. Seventy-two h after paclitaxel treatment, cells were analyzed by MTT assay. The percentage of cell growth was calculated by defining the absorption of cells not treated with paclitaxel as 100%. Error bars, SDs. All data points have error bars within the SD range of 1–3%. Cell lysates of each transfectant and parental cell lines were subjected to 12% SDS-PAGE and blotted with anti-p185 and anti-E1A antibodies. Equal loading was confirmed by probing the same SDS-PAGE gel with anti-actin antibody. HER-2/neu expression signal intensity was adjusted based on the original strength of the signal. The SKOV3.ip1 and 2774-c-10 nitrocellulose membranes were exposed at different time points. A, the effects of E1A on the expression of HER-2/neu in a human ovarian cancer cell line. B, enhanced growth inhibition by paclitaxel was seen on the viable fractions of the E1A-transfected HER-2/neu-overexpressing SKOV3.ip1 cell lines, but not on those of the control cell lines. C, no enhanced growth inhibition by paclitaxel was seen on the viable fractions of either the E1A-transfected low-HER-2/neu-expressing 2774-c-10 cell line or the control cell lines.
RESULTS

Correlation between Enhanced Sensitivity to Paclitaxel and Low HER-2/neu Expression Level. To determine whether the E1A sensitization of human ovarian cancer cell lines to paclitaxel is related to HER-2/neu, the cytotoxicity of paclitaxel against human ovarian cancer cells expressing different levels of E1A and HER-2/neu-encoded p185 proteins was compared. To generate stable transfectants constitutively expressing E1A, the genomic adenovirus type 5 E1A gene was transfected into both HER-2/neu-overexpressing SKOV3.ip1 cells and low-HER-2/neu-expressing 2774-c-10 cells (Fig. 1A, right panels).

Two stably transfected SKOV3.ip1 lines in which HER-2/neu expression was constitutively repressed by expression of the genomic E1A gene [the previously established SKOV3.ip1-E1A2 cell line (25) and the newly established cell line SKOV3.ip1-E1A16] were used. The cell line SKOV3.ip1-Efs was established as a negative control of SKOV3.ip1-E1A transfectants by transfecting SKOV3.ip1 cells with pE1Ad343, a plasmid containing a 2-bp frameshift deletion in the E1A coding sequence (Fig. 1A, right panels).

Three stably transfected 2774-c-10 cell lines (2774-c-10-E1A3, 2774-c-10-E1A7, and 2774-c-10-E1A8) were also established. Because E1A down-regulates HER-2/neu overexpression through an indirect mechanism by inactivating a transcriptional coactivator, p300 (39), HER-2/neu expression may not be inhibited in a stable E1A transfection setting in the low-HER-2/neu-expressing cell line (18, 40). Consistent with previous reports (18, 40), no repression of HER-2/neu expression was observed in the 2774-c-10-E1A transfectants. By pooling 2774-c-10 cells transfected with the neomycin resistance gene, the cell line 2774-c-10-NP was established as a positive control (Fig. 1A, left panels).

HER-2/neu overexpression is correlated with resistance to paclitaxel, as shown in Fig. 1B. Chemosensitivity was monitored by MTT assay 72 h after paclitaxel exposure. Parental low-HER-2/neu-expressing 2774-c-10 cells were more sensitive to paclitaxel (0.01–10 μM) than were parental HER-2/neu-overexpressing SKOV3.ip1 cells. SKOV3.ip1-E1A2 and SKOV3.ip1-E1A16 cells, in which HER-2/neu overexpression was down-regulated by E1A, were more sensitive to paclitaxel (0.1 or 10 μM) than were the parental SKOV3.ip1 cells or the SKOV3.ip1-Efs cells. In the 2774-c-10 cells in which the HER-2/neu level was not affected by E1A, no difference in sensitivity to paclitaxel was found, regardless of the level of E1A expression (0.001 or 10 μM; Fig. 1C).

Because paclitaxel is a G2-M-phase-specific inhibitor (41, 42), a faster cell growth rate might have contributed to the paclitaxel-sensitive phenotype. However, all of the cell lines studied but one showed similar growth rates; the SKOV3.ip1-E1A cell line (SKOV3.ip1-E1A2 and SKOV3.ip1-E1A16) grew more slowly. Thus, as found with breast cancer cell lines,
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Enhancement of Paclitaxel-induced Apoptosis by Down-Regulation of HER-2/neu Expression. In addition to stabilizing microtubules (41), paclitaxel induces apoptosis as part of a cytotoxic mechanism (43–45). HER-2/neu overexpression confers the ability to resist the induction of apoptosis on cells (20). Therefore, we next examined the possibility that the enhanced sensitivity to paclitaxel seen in paclitaxel-resistant HER-2/neu-overexpressing SKOV3.ip1 cells was due to the rapid induction of apoptosis by E1A-induced down-regulation of HER-2/neu overexpression.

The series of SKOV3.ip1 cells were treated with paclitaxel (0.1 or 1 μM) for 33 h and then analyzed for DNA content by flow cytometry. Increased sub-G0 fractions were found in SKOV3.ip1-E1A cells, in which HER-2/neu overexpression was repressed by E1A. In comparison, the controls (parental SKOV3.ip1 and SKOV3.ip1-Efs cells) were arrested by paclitaxel at G2-M phase (41; Fig. 2). Both E1A-transfected and control low-HER-2/neu-expressing 2774-c-10 cells showed an increased sub-G0 fraction after treatment with paclitaxel (0.01 or 0.1 μM) for 24–33 h, regardless of E1A expression level (Fig. 2). This finding was further confirmed with the finding that the sub-G0 fraction correlated with the appearance of internucleosomal DNA fragmentation on agarose gels (Fig. 3), a relationship that is highly consistent with the induction of apoptosis. Together, these results indicate that enhanced sensitivity to paclitaxel by E1A is observed only in HER-2/neu-overexpressing cells.

Once it had been determined that apoptosis was induced in SKOV3.ip1-E1A cells in which HER-2/neu overexpression was down-regulated by E1A, the effects of E1A on the expression levels of two proapoptotic molecules, Bax and Bad, and two antiapoptotic molecules, Bcl-2 and Bcl-XL, were studied. Protein samples collected from the series of SKOV3.ip1 cell lines before and after treatment with paclitaxel (0.1 μM) were subjected to Western blot analysis. Analysis showed that the expression levels of these apoptotic regulators were not affected by E1A or HER-2/neu expression levels (Fig. 4). However, all bands of treated Bcl-2 and Bcl-XL did migrate more slowly than did the untreated bands; these bands probably represented phosphorylation of the molecules by paclitaxel, as described previously (45–47). Furthermore, there was an overall decrease in Bad expression level after paclitaxel treatment.

Partial Requirement for Caspase-3 Activation in Paclitaxel-induced Apoptosis in HER-2/neu-Down-Regulated Cells by E1A. Both caspase-3 and caspase-7 are known to be activated in paclitaxel-induced apoptosis (48, 49); therefore, we next examined the relationship between caspase activity and paclitaxel-induced apoptosis in HER-2/neu-down-regulated ovarian cancer cells by the E1A gene. Caspase-3 and caspase-7 cleave PARP (M₆₆,000) at the time of their activation. To determine whether the cleaved M₆₆,000 PARP fragment could be detected in a series of SKOV3.ip1 cell lines after treatment with paclitaxel (0.1 μM), protein samples were collected every 6 h after paclitaxel treatment and subjected to Western blot analysis. The cleaved PARP product appeared at 24 h in paclitaxel-treated SKOV3.ip1-E1A cells, but not in the parental
SKOV3.ip1 cells or in the control SKOV3.ip1-Efs cells (Fig. 5A).

We then examined whether either pro-caspase-3 or pro-caspase-7 was activated in this type of apoptosis by Western blot analysis. Twenty-four h after treatment with paclitaxel (0.1 μM), cleavage appeared with caspase-3, but not with caspase-7 (Fig. 5B). These findings suggest that caspase-3 was the principal type of caspase activated.

Finally, the possibility that caspase-3 is a prerequisite for paclitaxel-induced apoptosis in HER-2/neu-overexpressing cells was examined. The series of four SKOV3.ip1 cell lines was treated with the caspase inhibitor Z-VAD-FMK, a potent cell-permeable pan-caspase inhibitor, or Z-DEVD-FMK, which is also cell permeable and effectively inhibits only caspase-3 activity. After exposure to paclitaxel (0.1 μM), chromatin fragmentation was evaluated by Hoechst staining of nuclei. Paclitaxel-treated SKOV3.ip1-E1A cells, but not parental SKOV3.ip1 cells and control SKOV3.ip1-Efs cells, displayed extensive fragmented nuclei. When the SKOV3.ip1 series was pretreated with Z-VAD-FMK, nearly complete inhibition of paclitaxel-induced apoptosis was observed; however, when treated with Z-DEVD-FMK, only the SKOV3.ip1-E1A cell line showed significant (but incomplete) inhibition of apoptosis (Fig. 6).
Combined Paclitaxel and E1A Gene Therapy

From these experiments, it can be concluded that activation of caspase-3 contributes to E1A-mediated sensitization to paclitaxel-induced apoptosis in HER-2/neu-overexpressing ovarian cancer cells.

**Prolonging Survival of Nude Mice bearing HER-2/neu-overexpressing Ovarian Cancer Cells after Combined Paclitaxel and E1A Gene Therapy.** After establishing enhanced sensitivity to paclitaxel in HER-2/neu-down-regulated human ovarian cancer cells by E1A in vitro, the effects of the E1A gene on paclitaxel sensitivity of HER-2/neu-overexpressing human ovarian cancer cells was examined in vivo.

Female nude mice received i.p. injections of HER-2/neu-overexpressing human ovarian cancer SKOV3.ip1 cells. Five days later, they received i.p. injections of E1A plasmids complexed with E1A/DC-Chol complexes, and 48 h later, they received i.p. injections of paclitaxel (15 mg/kg). As controls, other nude mice received i.p. injections of either E1A/DC-Chol complexes alone weekly or paclitaxel (15 mg/kg) alone every third week for a period of 17 weeks or were not treated. Each of the four groups included five mice.

The mice treated with both the E1A/DC-Chol complex and paclitaxel survived significantly longer than those treated with either E1A/DC-Chol alone or paclitaxel alone (P = 0.00217; Fig. 7). There was no tumor formation in three of the five mice. These findings indicate that the E1A gene can, through the down-regulation of HER-2/neu expression, enhance the sensitivity of HER-2/neu-overexpressing human ovarian cancer cells to paclitaxel and thus induce rapid apoptosis. In nude mice bearing paclitaxel-resistant HER-2/neu-overexpressing ovarian cancer cells, a therapeutic effect is produced that consequently improves overall survival.

**DISCUSSION**

From this study of stable E1A transfectants of human ovarian cancer that have the same genetic background but express different levels of HER-2/neu and E1A, it can be concluded that down-regulation of HER-2/neu by E1A forces HER-2/neu-overexpressing human ovarian cancer cells that were originally resistant to paclitaxel to become paclitaxel sensitive. This finding is consistent with that from a previous study of HER-2/neu-overexpressing breast cancer cells (18). Moreover, studies of the paclitaxel-resistant HER-2/neu-overexpressing ovarian cancer xenograft animal model have revealed that combined treatment with both paclitaxel and E1A gene therapy prolongs overall survival in nude mice.

E1A apparently enhances the sensitivity of both cancer and noncancer cell lines to chemotherapeutic DNA-damaging agents such as the alkylating agents cisplatin or Adriamycin (29, 30, 50–52) and paclitaxel (18, 52). This enhanced sensitivity is partially caused by the induction of p53-dependent apoptosis by E1A-induced sensitization of the cells (29). Furthermore, this apoptosis requires stabilization of p53 expression by p19ARF (30, 53) or its human homologue, p14ARF (54). Other groups have reported sensitization to cisplatin via a p53-independent mechanism (51). In the study reported here, cell lines SKOV3.ip1 and 2774-c-10 were used; in both cell lines, p53 is deleted or mutated.

It appears that HER-2/neu overexpression may be a dominant factor in conferring paclitaxel resistance on the cells in the experimental model used. This may agree with the previous study of paclitaxel-treated stable transfecteds of mouse embryo fibroblasts having the same genetic background but expressing different levels of HER-2/neu, suggesting that paclitaxel sensitivity is HER-2/neu dependent (18). However, further study is needed to determine whether this resistance is dependent on HER-2/neu overexpression and whether other mechanisms may also be involved.

The results from the studies conducted suggest that the enhancement of chemosensitivity to paclitaxel by E1A-induced down-regulation of HER-2/neu overexpression may allow the development of novel therapeutic approaches for HER-2/neu-overexpressing ovarian cancers, cancers known to have poor prognosis. Other investigators have modulated the HER-2/neu pathway by either targeting the extracellular domain by the antibody (55) or inhibiting the intracellular tyrosine kinase domain by means of a tyrosine kinase inhibitor (56, 57), which can enhance the sensitivity of cells to chemotherapeutic agents. A recent clinical trial in patients with HER-2/neu-overexpressing breast cancer revealed that the combination of paclitaxel and humanized anti-HER-2/neu antibody (Herceptin), when com-

![Fig. 6 Partial inhibition of paclitaxel-induced apoptosis by a cell-permeable caspase-3 inhibitor in HER-2/neu-overexpressing ovarian cancer cells. Cells were pretreated for 1 h with either the pan-caspase inhibitor (Z-VAD-FMK) or the caspase-3 inhibitor (Z-DEVD-FMK). Paclitaxel (0.1 μM) was then applied, and after Hoechst staining, the cells were examined for signs of apoptosis, that is, changes in cell morphology and chromatin condensation. The number of total cells and the number of apoptotic cells were counted in four areas under the high-power field of a fluorescent microscope. Three independent experiments were conducted. The percentage of apoptotic cells was calculated in relation to the total number of untreated control cells, which was set at 100%. Error bars, SDs.

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pared with paclitaxel alone, produced a significant increase in the tumor response rate (58).

In the recently completed Phase I trial of E1A gene therapy, a trial whose rationale was based on E1A being a significant tumor suppressor in HER-2/neu-overexpressing cancers (26, 27, 59), the E1A gene was delivered into either the thoracic or the peritoneal cavity of patients with HER-2/neu-overexpressing breast or ovarian cancers via the DC-Chol gene delivery system. In that trial, it was shown that the E1A gene could be transsected into cancer cells and that HER-2/neu is down-regulated in cancer cells after E1A gene delivery. Therefore, after completing the Phase II trial of E1A gene therapy alone, a Phase II trial for combined E1A gene therapy and paclitaxel treatment is being planned for the future.

Whereas rigorous testing of this new concept of combined E1A gene therapy and paclitaxel treatment in animal models is still needed, the findings reported strongly suggest that the concept may soon provide therapeutic benefits to patients with HER-2/neu-overexpressing ovarian cancers in the form of a Phase II trial.

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