Adenovirus E1A Expression Enhances the Sensitivity of an Ovarian Cancer Cell Line to Multiple Cytotoxic Agents through an Apoptotic Mechanism

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ABSTRACT

The introduction of adenovirus 5 E1A into the SKOV3ip1 ovarian cancer cell line was shown previously to suppress HER2/neu expression and reduce the malignant potential of these cells (Yu et al., Cancer Res., 53: 891–898, 1993). In this report, we show that reduction of p185 in cells stably expressing E1A protein was coincident with increased sensitivity to cytotoxic agents. The LD_{50} of cisplatin was reduced 6-fold, and the LD_{50} of paclitaxel and doxorubicin was reduced 10-fold in E1A-expressing cells compared with control cells. The growth of SKOV3ip1 and control cells was unchanged in the presence of 150 ng/ml of tumor necrosis factor-α, whereas the growth of E1A-expressing cells was reduced by 30 to 40%. When we used a physiologically obtainable concentration of paclitaxel (0.5 μM), DNA laddering consistent with apoptotic cell death was seen after a 24-h exposure in the E1A-expressing cells, whereas laddering and DNA fragmentation were only detected in DNA from control cells after longer exposure (48 h) at a 20-fold higher concentration of paclitaxel. The SKOV3ip1 cells do not express p53 protein; hence, the induction of apoptosis by paclitaxel is through a p53-independent pathway. Despite their diverse mechanisms of action, the cytotoxic effects of cisplatin, doxorubicin, paclitaxel, and tumor necrosis factor-α were enhanced by the expression of E1A proteins in the SKOV3ip1 ovarian cancer cells. This suggests that these agents share a common final pathway of cell killing, which may represent a potential therapeutic target in resistant ovarian cancers.

INTRODUCTION

The adenovirus 5 early region 1a (E1A) gene products are multifunctional transcriptional regulators capable of interacting with and perturbing the function of key regulators of cell growth. The primary function of the E1A gene products is to activate other adenoviral genes during a permissive viral infection by modifying the transcriptional apparatus of the host cell. E1A functions in concert with EIB to regulate the expression of viral genes in transformed cells (1), although expression of E1A alone predisposes such cells to apoptosis. E1A-induced apoptosis has been reported to be dependent on the presence of wild-type p53 protein (2) and can be inhibited by EIB proteins (2, 3) or bcl-2 (4). E1A expression can sensitize certain cells to apoptosis induced by various cytotoxic agents. Induction of E1A expression has been shown to increase the susceptibility of myeloid leukemia cells to lysis by TNFα (5). Transfection of a fibroblast cell line with E1A sensitized these cells to apoptosis induced by ionizing radiation and by chemotherapeutic agents that have widely divergent mechanisms of action. This cell death was regarded as p53 dependent, because p53 null cells did not show enhanced sensitivity after E1A transfection (6).

Systemic chemotherapy is the standard treatment for metastatic ovarian cancer. Despite improved median survival for patients with advanced stage disease treated with cisplatin and, more recently, paclitaxel, the development of resistance remains a major obstacle to improving overall survival. The initial response rates to these agents can be as high as 80–90%, but the majority of patients relapse (7). Response rates to second-line agents are 25–30%, with few durable responses (8). It appears that the development of resistance to the first-line agents cisplatin and paclitaxel is indicative of a general unresponsiveness to further cytotoxic therapy. Much attention has been focused on developing new agents with unique mechanisms of action, but chemotherapy resistance in ovarian cancer may not be occurring at the level of the drug-target interaction. Instead, a downstream process may be involved. A defect or alteration in the apoptotic pathway could lead to resistance to multiple cytotoxic agents. Indeed, defects in the apoptotic pathway have been shown to produce treatment-resistant tumors in vivo (9). Restoration or enhancement of apoptosis in drug-refractory tumor populations could potentially prevent or reverse drug resistance.

In this study, we report that the human ovarian cancer cell line, SKOV3ip1, was found to be relatively resistant to various cytotoxic agents (cisplatin, paclitaxel, doxorubicin, and TNFα).

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The abbreviations used are: TNF, tumor necrosis factor; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FACS, fluorescence-activated cell sorting; DPA, diphenylamine.
The introduction of the E1A gene, which we showed previously could suppress expression of HER2/neu and reduce malignant growth potential of these cells (10), resulted in increased sensitivity to all of these agents, which act through different modes of action. For paclitaxel treatment, the increased sensitivity was associated with enhanced induction of DNA fragmentation, one indicator of apoptosis. The SKOV3 and variant cell lines were all p53 null, and thus the E1A-enhanced apoptosis observed must occur through a p53-independent mechanism.

**MATERIALS AND METHODS**

**Cell Lines.** The human ovarian cancer cell line SKOV3 was obtained from the American Type Culture Collection. The SKOV3ip1 cell line was derived from the ascites of a nude mouse injected with the original cell line (10). As described previously, this cell line was transfected with either the E1A-expressing plasmid (pE1A) or pE1A-d1343, which contains a two-base frameshift deletion in the E1A coding region. The transfection mixtures also contained pSV2neo plasmid to confer resistance to G418 (10). The cell line and derivatives were maintained in monolayer culture in Eagle’s MEM supplemented with 5% FBS, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 1 mM L-glutamine, 50 units/ml penicillin G, 50 μg/ml streptomycin sulfate and MEM vitamin supplement (medium and supplements from Life Technologies, Inc., Grand Island, NY). The transfected cells were maintained in medium containing 800 μg/ml G418 (Life Technologies, Inc.). Cells were harvested by incubation with 0.25% trypsin-0.02% EDTA to harvest the cells were initially plated in 96-well plates, as above, in MEM with 5% FBS. After 1-5 days incubation, the relative cell number was determined using the MTT assay (11). To assess the serum dependence of the cell lines, the cells were initially plated in 96-well plates, as above, in MEM with 5% FBS. After 24 h incubation, the medium was aspirated, the wells were washed twice with serum-free MEM, and medium was supplemented with 0 to 5% FBS added. After a 6-day incubation period, the relative cell numbers were determined using the MTT assay.

**Comet Sensitivity Assays.** Cells were plated in 96-well plates, 5 × 10³ cells/well. After 24 h incubation, the medium was aspirated and replaced with MEM with 5% FBS containing a range of concentrations of rHu-TNF-α (Genzyme, Cambridge, MA), cisplatin (Bristol-Myers Oncology Division, Syracuse, NY), doxorubicin (Ben Venue Laboratories Inc., Bedford, OH), or paclitaxel (Sigma Chemical Co., St. Louis, MO), and incubated for an additional 72 h. Relative numbers of viable cells remaining were determined using the MTT assay. In assays for sensitivity to TNF-α, the mouse L929 fibroblast cell line served as a positive control, because these cells are exquisitely sensitive to this cytokine (12).

**Western Blot Analyses for E1A and p53 Proteins.** Protein lysates were prepared from cultures of cells in the logarithmic phase of growth. Aliquots of 10 μg of protein were separated electrophoretically on 10% SDS-polyacrylamide gels and transferred to nitrocellulose filters. For E1A expression, the filters were incubated for 1 h at room temperature with mouse monoclonal antibody M73 (Santa Cruz Biotechnology, Santa Cruz, CA). For p53 protein analysis, the filters were hybridized with the DO-1 mouse monoclonal antibody (Santa Cruz Biotechnology), which recognizes the NH₂-terminal epitope of wild-type and mutant human p53 protein. The filters were incubated with anti-mouse horseradish peroxidase-conjugated antibody and developed with ECL detection reagents (Amersham Corp., Arlington Heights, IL). To confirm equal loading and transfer of protein, the filters were stripped and re-probed with a rabbit anti-actin antibody (Sigma Chemical Co.) and anti-rabbit horseradish peroxidase-conjugated antibody (Amersham). Densitometric calculations of the intensity of bands corresponding to specific proteins were performed using a Molecular Dynamics Personal Densitometer.

**FACS Analysis.** Cells grown to 70 to 80% confluence were harvested with trypsin and EDTA and suspended in PBS with 1% FBS at 1 × 10⁶ cells per ml. Monoclonal anti-human p185 (Ab5, Oncogene Science, Uniondale, NY) was added at a final dilution of 1:250 and incubated at 4°C for 30 min. The cells were washed with PBS containing 2% FBS and 0.02% sodium azide, then incubated with FITC-conjugated goat F(ab′)2 anti-mouse IgG (Biosource, Camarillo, CA) at 1:20 dilution, at 4°C for 30 min. The cells were washed extensively and fixed in 1% paraformaldehyde in PBS. FACS analysis was performed on an EPICS Profile Cell Sorter (Coulter, Hileah, FL) with a 525-nm band pass filter to detect FITC and gated on forward versus side scatter to exclude debris, dead cells, and clumps. Analysis was based upon cursors set at 2% for isotype-matched negative controls.

**DNA Laddering.** Cells were plated in 150-cm² tissue culture flasks to 70% confluence, and paclitaxel was added to the medium at the calculated LD₉₀ doses, 0.5 μM for E1A17 cells and 10 μM for EFS cells. After 24 h incubation, the cells were harvested with trypsin-EDTA and counted. A portion of the cells was used for the DPA assay (see below). Aliquots of 10⁶ cells were suspended in 0.3 ml of lysis buffer (1% sarcosyl, 0.01 M EDTA, and 10 mg/ml RNase A in PBS) and incubated for 4 h at 37°C. Proteinase K was added to a final concentration of 100 μg/ml and incubated at 50°C for 12 h. High molecular weight DNA was pelleted by high speed centrifugation, and the supernatant was extracted twice with equal volumes of phenol:chloroform:isoamyl alcohol (25:24:1). Following the addition of 1/10 final volume of 2 mM sodium acetate and 2 volumes of 100% ethanol to the aqueous supernatant and freezing at 70°C for 2 h, the precipitated DNA was pelleted, washed with 70% ethanol, and air dried. The DNA was then dissolved in 50 μl of TE buffer (10 mM Tris-1 mM EDTA) and separated electrophoretically in a 1% agarose/ethidium bromide gel at 20 V for 15 h. The gel was visualized under UV illumination and photographed.

**Diphenylamine Reaction.** The DPA reaction was used to quantify DNA fragmentation. In this assay, fragmented DNA released from the nuclei of lysed cells is separated from intact chromatin by centrifugation. The intact and fragmented DNA is quantified by measuring deoxyribose using the DPA colorimetric assay (13). The E1A17 and EFS cells were treated with paclitaxel for up to 72 h, as described above, and harvested with trypsin-EDTA. Aliquots of 5 × 10⁶ cells in 0.5 ml of PBS were
pelleted, and the supernatant fraction (S) was saved. The pelleted cells were lysed with 0.5 ml 0.2% Triton X-100 in TE buffer at pH 7.4 and centrifuged, and this supernatant fraction (T) was saved. The pellet fraction (B) was resuspended in 0.5 ml lysis buffer, and 0.5 ml of 10% trichloroacetic acid was added. After incubation at 4°C for 18 h, this mixture was centrifuged, and the pellet was suspended in 80 μl of 5% trichloroacetic acid, followed by incubation at 90°C for 20 min. This solution was centrifuged, and 40-μl aliquots of the supernatant and the S and T fractions were transferred to 96-well plates. To each aliquot, 80 μl of DPA solution (150 mg DPA in 10 ml of glacial acetic acid with 150 μl of sulfuric acid and 50 μl of acetaldehyde (16 mg/ml)) were added. The plates were incubated at room temperature for 16 h. The proportion of fragmented DNA was calculated from absorbance readings at 600 nm using the formula:

\[
\text{% fragmented DNA} = \frac{S + T}{S + T + B} \times 100
\]

RESULTS

SKOV3ip1 Transfectants Stably Express E1A Protein.
Western blot analysis demonstrated the expression of E1A protein in the E1A17 cells (Fig. 1). Because E1A expression alone is known to induce apoptosis, we were concerned that this would lead to selective loss of the E1A-expressing cells over time. The immunoblots showed that E1A proteins were expressed up to tissue culture passage 21, although with a reduction in protein expression (relative to levels of actin in the same samples) of 65% between passages 7 and 13. In cells harvested after passage 21, the E1A protein level was only 10% of that found in cells of passage 7. Based on these results, all experiments used the E1A17 cells at passage levels between 7 and 12. A second E1A transfectant (E1A19) that initially demonstrated a phenotype similar to E1A17 lost expression of E1A proteins after tissue culture passage 15. This loss led to the reversion to the original SKOV3ip1 phenotype, with high p185 expression and relative insensitivity to TNF-α, paclitaxel, cisplatin, and doxorubicin (data not shown). Because these cells did not express E1A in a stable manner, they were not used in additional experiments.

FACS Analysis of p185 Expression. FACS analysis can measure the relative expression of cell surface antigens and determine the percentage of the population expressing the antigen. SKOV3 is known to express high levels of the proto-oncogene HER2/neu (14), and we show in Fig. 2 the expression of the p185 product of this gene, as measured by FACS analysis. The mean fluorescence values shown are normalized to the original SKOV3 cell line. As reported previously (10), the more aggressive variant SKOV3ip1 expresses 48% more p185 on the cell surface. E1A expression reduces the level of p185 by 72% compared with the EFS cells. This reduction was maintained over multiple passages (measured up to passage 20), consistent with the presence of E1A protein in the E1A17 cells.

p185 expression in the SKOV3 cells was homogeneous, with 98–100% of cells of all of the derivative lines expressing the cell surface receptor. This is important as E1A expression might, in theory, lead to selection of cells that lack p185, resulting in a mixed population of cells that would be revealed as a biphasic curve on FACS analysis and less than 100% of cells expressing the antigen. The presence of a mixed population could result in misleading conclusions from the chemosensitivity assays. Using p185 expression as a marker, it would appear
that transfection of E1A into the SKOV3ip1 cells did not give rise to a heterogeneous population in the E1A17 cell line.

**Western Blot Analysis of p53.** SKOV3 cells have previously been reported to lack p53 (15). Establishing the p53 status of our cell line was important for the principal reason that E1A-mediated apoptosis has been reported to act through a p53-dependent pathway (2). To confirm reports of the p53 null status of the SKOV3 cell line and to ensure that these cells and the variants of the SKOV3ip1 line were not contaminated with other cells (16), Western blot analysis was used to detect p53 proteins. The DO-1 antibody used recognizes both wild-type and mutant human p53, but as shown in Fig. 3, none of the variants of SKOV3 express p53 protein. Controls shown are human cancer cell lines that express mutant or wild-type p53 or are p53 null [respectively, a clone of A375 melanoma (17), MCF-7 (Lane 6), and SAOS-2 (Lane 7)]. Total protein was isolated from each cell line, prepared in Laemmli lysis buffer, and then separated in a 10% SDS-PAGE gel (10 µg/lane) and electroblotted onto a nitrocellulose filter. The filter was reacted with a monoclonal antibody to human p53 protein and then stripped and reprobed with an antibody to actin.

![Western blot analysis of p53 protein in SKOV3 (Lane 1), SKOV3ip1 (Lane 2), EFS (Lane 3), E1A17 (Lane 4), A375C15 (Lane 5), MCF-7 (Lane 6), and SAOS-2 (Lane 7). Total protein was isolated from each cell line, prepared in Laemmli lysis buffer, and then separated in a 10% SDS-PAGE gel (10 µg/lane) and electroblotted onto a nitrocellulose filter. The filter was reacted with a monoclonal antibody to human p53 protein and then stripped and reprobed with an antibody to actin.](image)

**Growth Effects of E1A Expression in Vitro.** Because E1A expression can affect the proliferation of many cell types (20), the *in vitro* growth rate of the E1A17 cells was compared with that of the other SKOV3 variants. Previous studies had shown that E1A expression in the SKOV3ip1 cells led to reduced growth rate, decreased [3H]thymidine incorporation, and decreased colony-forming efficiency in semisolid agarose (10). Growth curves of the SKOV3, SKOV3ip1, and transfected cell lines confirmed the reduced growth rate of the E1A-expressing cells compared with the controls (Fig. 4). E1A expression resulted in an approximately 50% increase in the doubling time compared with the control EFS cell line.

Serum deprivation has been shown to induce apoptosis in cells expressing E1A proteins (21). To establish the degree of serum dependence caused by E1A expression in SKOV3ip1 cells, cells were grown in a range of serum concentrations (Fig. 5). E1A17 cells grew minimally in serum-free medium and showed a marked increase in growth with increased serum concentrations, with an 11-fold increase in the number of cells grown in medium with 5% FBS compared with serum-free MEM. In contrast, the EFS cells grew relatively well in serum-free MEM with only a 2-fold increase in cell number in the wells containing 5% FBS (Fig. 5). As a consequence, all experiments comparing the growth and chemosensitivity of the SKOV3 cells and transfectedants were performed in medium with 5% FBS.

**In Vitro Chemosensitivity.** To assess the effects of E1A expression on the sensitivity of the SKOV3 cells to cytotoxic agents, dose-response curves were generated for TNF-α, cisplatin, doxorubicin, and paclitaxel. As shown in Fig. 6, E1A expression led to an approximately 6-fold reduction in the LD$_{50}$ of cisplatin and a 10-fold reduction in LD$_{50}$ of doxorubicin and paclitaxel. This difference cannot be due to an E1A growth effect, for if anything, the growth-reducing effects of E1A should decrease chemosensitivity (22). SKOV3 and SKOV3ip1 cells had dose responses to these drugs that were similar to the control EFS cells (data not shown). SKOV3 has been reported to be insensitive to TNF-α (23), a finding confirmed in our results. After over 72 h of exposure at 150 ng/ml, there was no growth inhibition of SKOV3, SKOV3ip1, or EFS cells. However, the E1A17 cells showed a 30–40% inhibition of growth in repeated assays (Fig. 6).
E1A-enhanced Induction of Apoptosis. DNA laddering is characteristic of the internucleosomal DNA cleavage that occurs during apoptosis. Exposure to cytotoxic agents has been shown to induce DNA laddering in a number of cancer cell lines (24), although this is not a consistent finding following exposure to cisplatin (25). Because paclitaxel does not directly interact with DNA or function as a cytotoxic agent through induction of DNA adducts or strand breaks (26), we chose this agent to determine if the enhanced cytotoxicity induced in the E1A-expressing cells was associated with an alteration in the apoptotic pathway. In vitro cytotoxicity studies had shown the LD_{90} dose of paclitaxel for E1A17 to be 0.5 \mu M. For EFS cells, the maximal cell kill (80%) resulted from exposure to 10 \mu M paclitaxel for 72 h. Using these doses to produce an approximately comparable degree of cell kill, the E1A17 and EFS cells were exposed to paclitaxel for 24 h, and the DNA was examined for laddering. The 24-h exposure time was chosen because the cells showed significant morphological changes at this time (data not shown).

No laddering was detected in the DNA from untreated E1A17 or EFS cells, and even after 24 h exposure to 20 \mu M paclitaxel (data not shown), no ladder formation was detected in the DNA from the control transfected line. The DNA from E1A17 cells, however, showed a classic ladder pattern after 24 h exposure to 0.5 \mu M paclitaxel (Fig. 7). Longer exposure of EFS cells to paclitaxel resulted in ladder formation in samples collected at 60 h after addition of the drug (data not shown). A more sensitive and objective measure of DNA fragmentation was obtained with the DPA reaction, shown in Fig. 8. Paclitaxel (10 \mu M) exposure for 72 h resulted in between 30 and 40% DNA fragmentation in the EFS cells, whereas greater than 75% of the DNA was fragmented in E1A17 cells treated with 0.5 \mu M paclitaxel for 72 h. Thus, the expression of E1A proteins in the SKOV3ip1 cells significantly decreased the dose of paclitaxel required to induce apoptosis and also facilitated induction of apoptosis after a shorter exposure time.

The results of the DPA reaction show that in the E1A17 cells, there was a baseline level of apoptosis (approximately 10% fragmentation) in the absence of paclitaxel and not detected in the DNA ladders (Fig. 7). This was higher than the baseline level of fragmentation (1–2%) in the EFS cells (Fig. 8). This could explain the reduction in the level of E1A proteins in these cells over time in tissue culture (Fig. 1), presumably from the gradual loss of cells with the introduced gene.

DISCUSSION

The relative insensitivity of recurrent ovarian cancer to cytotoxic therapy is well known to the physician. Failure of first-line therapy is predictive of poor response to subsequent therapy, regardless of the agent. On a cellular level, it is possible to imagine a heterogeneous primary tumor population containing cells that respond to cytotoxic agents and others that do not. Drug resistance can result from multiple factors, including decreased drug accumulation, increased DNA repair, and cellular drug inactivation (27, 28). Another cause of resistance may be a deranged apoptosis pathway (9). Thus primary cytotoxic therapy will kill responsive cells, and cells with a defect in the apoptotic response may selectively survive. This would result in tumors resistant to multiple agents, a finding all too common in the clinic. Modulation of the apoptotic pathway thus represents a
logical target for therapeutic intervention in both primary and chemorefractory ovarian cancer.

In this study, we show that transfection of an ovarian cancer cell line with the adenovirus E1A gene results in increased sensitivity to multiple cytotoxic agents. The SKOV3ip1 cell line was shown to be relatively resistant to various agents and to undergo paclitaxel-induced apoptosis only at high dose of drug and after prolonged exposure. After introduction of E1A, apoptosis was observed at a 20-fold lower concentration of paclitaxel and after a shorter exposure time. The dose and exposure time that resulted in apoptosis in the E1A-expressing cells were consistent with physiological obtainable conditions (29). Because of the diverse mechanisms of action of the agents used and because E1A proteins are reported to increase cellular susceptibility to apoptosis, we hypothesized that E1A acts downstream of the drug-target interactions, potentially at the level of apoptosis induction.

The ability of E1A gene products to induce apoptosis is well established (2–4). In some of these studies, E1A-induced apoptosis was shown to be p53 dependent (30) and related to the ability of the E1A protein to stabilize p53 (31). The increased

![DNA ladder in E1A-expressing SKOV3ip1 cells exposed to paclitaxel. Cells of the EFS line and E1A17 line were exposed to paclitaxel at the LD₅₀ dose (10 μM and 0.5 μM, respectively) for 24 h. The cells were harvested, and high molecular weight DNA was extracted from 10⁶ cells. The DNA was separated electrophoretically in a 1% agarose/ethidium bromide gel at 20 V for 15 h. The gel was viewed under UV illumination and photographed.](image)

Fig. 7 DNA ladder in E1A-expressing SKOV3ip1 cells exposed to paclitaxel. Cells of the EFS line and E1A17 line were exposed to paclitaxel at the LD₅₀ dose (10 μM and 0.5 μM, respectively) for 24 h. The cells were harvested, and high molecular weight DNA was extracted from 10⁶ cells. The DNA was separated electrophoretically in a 1% agarose/ethidium bromide gel at 20 V for 15 h. The gel was viewed under UV illumination and photographed.

![DNA fragmentation in EFS and E1A17 cells exposed to paclitaxel.](image)

Fig. 8 DNA fragmentation in EFS and E1A17 cells exposed to paclitaxel (10 μM and 0.5 μM, respectively) for up to 72 h. Fragmented DNA released from the nuclei of paclitaxel-treated cells was measured using the DPA colorimetric assay and expressed as a percentage of the total DNA in the sample. The results shown are the means from a representative of three repeat experiments; bars, SE. □, EFS control; ■, EFS treated with paclitaxel; △, E1A17 control; ♦, E1A17 treated with paclitaxel.

half-life and amount of wild-type p53 results in enhanced growth arrest and apoptosis. E1A expression has been shown previously to enhance in vitro cytotoxicity to ionizing radiation, chemotherapy, and TNF-α (32–35), and for certain cell types, this sensitization was dependent on the presence of p53 (6).

In contrast, our results and those of several other studies demonstrate enhancement of apoptosis and increased chemosensitivity by E1A in the absence of p53 (33, 34). Sanchez-Prieto et al. (33, 36) showed no correlation between p53 levels and the sensitivity of E1A-transfected keratinocytes and cancer cell lines to DNA-damaging agents. Their study with mouse keratinocytes used mutants of E1A that differed in potential for binding to cellular proteins implicated in cell cycle control, including p60, pbo7, plo7, and p300. The results suggested that apoptosis induced by different agents may involve distinct cellular targets and can be unrelated to the amount of p53 present. The involvement of additional targets in the cell cycle regulatory pathway was investigated in a study by Nakajima et al. (37) in which E1A-induced apoptosis was preceded not only by a rise in p53 but also by a precipitous drop in topoisomerase IIα. These results suggested that E1A can activate or induce components in the ubiquitin proteolysis pathway responsible for degrading topoisomerase IIα (37). This enzyme serves to maintain the integrity of the nuclear matrix, and a decreased level may make DNA more vulnerable to endonuclease activity. E1A may be targeting topoisomerase IIα and/or other cell cycle regulatory molecules in the SKOV3ip1 cells, such as products of the retinoblastoma gene family or E2F (38, 39), aspects that remain open to investigation. Although E1A interactions with p300 and pRb have been linked to perturbation of cell cycle and apoptosis induction, a recent study showed that mutants of E1A lacking pRb and p300 binding affinity could still induce apoptosis. Thus, either pRb and p300 interactions with E1A are not required for apoptosis, or there are redundant apoptotic pathways initiated by or involving E1A (40). Regardless of the identity of the ultimate cell targets and pathways involved, it is evident that E1A-induced apoptosis and enhanced cytotoxicity can occur by both p53-dependent and
-independent pathways. Based on these studies and the range of agents whose activities are enhanced by expression of E1A, this gene may represent a potential chemomodulator both for treating primary ovarian cancer and for reversing chemoresistance in refractory ovarian cancer.

The ability to induce apoptosis in the absence of wild-type p53 in an ovarian cancer cell line is significant. p53 alterations are common in epithelial ovarian cancer, with a frequency of over 50% in advanced stage disease (41). Alterations in p53 in ovarian cancer have been shown to correlate with overall prognosis and with response to chemotherapy (42). The high rate of p53 alterations in advanced ovarian cancer means that a form of chemomodulation that acts independently will be more applicable than one that is dependent on the presence of wild-type p53. In this study, we demonstrate E1A-enhanced cytotoxicity in the absence of p53, although whether E1A can act as effectively in an ovarian cancer cell expressing mutated p53 gene is unknown at present.

The SKOV3ip1 cell line overexpresses the M, 185,000 product of HER2/neu (p185), and in a previous report, we showed that E1A suppressed the level of p185 and the malignant growth potential of the cells (10). Recent results suggest that regulation of HER2/neu by E1A may involve interactions with p300 (43). Various studies with different cancer cell lines suggest that increased expression of the p185 growth factor receptor is associated with reduced sensitivity to various cytotoxic agents, including those used in our study (23, 44, 45). Similarly, down-regulation of p185 in SKOV3 cells by introduction of a plasmid construct encoding an intracellular single chain antibody resulted in increased sensitivity to cisplatin (46). The cumulative results from a number of studies suggest a link between p185-initiated signal transduction pathways and the repair of cisplatin-induced DNA damage (47–49). Another link between p185 and sensitivity to paclitaxel is the finding that breast cancer cells transfected with c-erbB2/neu show reduced cdc2/cyclin B activation, concomitant with reduced sensitivity to paclitaxel (50). Thus, signaling through p185 may reduce paclitaxel-induced killing by inhibiting cdc2/cyclin B activation (51). Although experimental evidence suggests that p185 plays a role in responses to chemotherapeutic agents, whether this is by a single or multiple mechanisms is not yet known. E1A expression enhanced the sensitivity of SKOV3ip1 cells to various agents, yet we cannot distinguish whether this was associated with the reduction of p185 or due to a p185-independent mechanism. The latter possibility is supported by the observations that introduction of E1A into several cell lines that did not express p185 resulted in increased sensitivity to etoposide and cisplatin (34). Recent reports have shown that the in vivo introduction of E1A can be achieved with an adenoviral vector, or in liposomes, resulting in the suppression of p185 and increased survival of nude mice injected with SKOV3ip1 cells (52, 53). Based on our results and those of others, this mode of gene therapy may be expected to also enhance in vivo chemosensitivity, possibly regardless of the p185 status of the tumor.

Clearly, multiple pathways are involved in the regulation of cell growth and death and also responses to cytotoxic agents. Within one tumor, it is likely that there will be heterogeneity in sensitivity to a particular form of therapy. Targeting sites downstream of drug-substrate interactions may overcome both intratumor heterogeneity and the potential for iatrogenic drug resistance.

Novel approaches for therapy are definitely warranted considering the relative plateau that has been reached in the treatment of ovarian cancer. Despite the prolonged disease-free survival times seen with the introduction of paclitaxel (54), overall relapse and death rates will probably remain unchanged. By targeting the final common pathways through which multiple agents act, we may be able to make a more significant impact on the natural history of this disease. E1A-based therapy, with the goal of increasing chemosensitivity to a range of therapeutic agents in addition to the modulation of growth, represents a potential new approach for improving the treatment of ovarian cancer.

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2024 Adenovirus E1A and Chemosensitivity


Adenovirus E1A expression enhances the sensitivity of an ovarian cancer cell line to multiple cytotoxic agents through an apoptotic mechanism.

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