Suppression of Ovarian Cancer Cell Tumorigenicity and Evasion of Cisplatin Resistance Using a Truncated Epidermal Growth Factor Receptor in a Rat Model

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Abstract
The overexpression of the epidermal growth factor receptor (EGFR) is associated with a poor prognosis in ovarian cancer. The dominant-negative EGFR (EGFR-DNR) is a truncated receptor that lacks the tyrosine kinase domain and is devoid of signaling capability. This study tested the effects of an EGFR-DNR approach in ovarian cancer cells. NuTu-19, a rat ovarian cancer cell line was rendered resistant to cisplatin. Both NuTu-19 and resistant cells were infected with a retroviral vector containing the EGFR-DNR. NuTu-19 and NuTu-DNR (NuTu-19 cells expressing the EGFR-DNR) were injected into Fisher 344 immunocompetent rats. Western blot analyses were used to assess signal transduction pathways. All rats injected with NuTu-DNR cells remained healthy following tumor injection. In contrast, 100% of the rats injected with the NuTu-19 and NuTu-Sham (NuTu-19 cells expressing an empty vector) died of disease progression at the end of 15 weeks (P = 0.00009). On Western blot analysis, both NuTu-19 and NuTu-Sham cells showed a strong activation of mitogen-activated protein kinase (MAPK) after exposure to EGF. Cisplatin-resistant cell lines showed an enhanced EGF stimulatory effect via the MAPK pathway compared with parental cells. The EGFR-DNR significantly reduced the ability of EGF to induce cell signaling through the MAPK pathway. Lastly, the EGFR-DNR can partially reverse cisplatin resistance in drug-resistant cells. The EGFR-DNR approach suggests that EGFR confers a growth advantage to NuTu-19 cells in vitro. Thus, EGFR blockade may ultimately prove to be a useful therapeutic tool in the treatment of cisplatin-sensitive and cisplatin-resistant ovarian cancers.

Introduction
Ovarian cancer is the leading cause of mortality from gynecologic cancers in the United States, resulting in ~14,500 deaths annually. With an incidence of 26,600 cases per year, ovarian carcinoma will develop in 1 in 70 women in their lifetime, and one woman in 100 will die of complications associated with this disease. Despite good initial responses to chemotherapy, 75% of women ultimately die of complications associated with disease progression (1, 2). Consequently, there is a strong impetus to investigate new therapies to improve the outcome of patients afflicted with this deadly disease. In fact, the NIH Consensus Statement on ovarian cancer advocated on the importance of innovative ovarian cancer research focusing on “molecular targets, agents to overcome resistance, and drugs that inhibit signal transduction pathways.”

Cisplatin-based chemotherapy has significantly improved the survival of advanced ovarian cancer patients. Although cisplatin is one of the essential chemotherapeutic drugs in the first-line treatment of ovarian cancer, resistance to cisplatin at the onset of treatment or at relapse is the most significant cause of treatment failure in ovarian cancer (1). Some of the mechanisms of resistance involve events such as alterations in drug transport, enhanced DNA repair, intracellular detoxification with glutathione, and changes in signal transduction (12). The EGFR and its signaling pathways are involved with the mechanism of cisplatin resistance. Cells that are resistant to cisplatin have an altered response to the EGF ligand and enhanced activation of protein kinase (13, 14). To recapitulate drug-resistant cells, we isolated cisplatin-resistant cell variants, NuTu-CPR (in vitro selection) and NuTu-ASC (in vivo selection),
from the parental NuTu-19 ovarian cancer cell line. This selection enabled us to evaluate the characteristics associated with drug-sensitive to drug-resistant phenotypes. These studies further investigated the role of EGFR as a mediator for drug resistance and the effect of the EGFR-DNR on drug-resistant cells.

**Materials and Methods**

**Generation of expression vectors.** Cloning of the EGFR cDNA and generation of the corresponding truncated EGFR construct has been previously described (6). The truncated EGFR (EGFR-DNR) consists of the extracellular and transmembrane regions of the EGFR but is devoid of its intracellular kinase domain (15–17). EGFR-DNR was subcloned into a Moloney murine leukemia virus (MMLV)–based retroviral vector CH#2, yielding pCH/HER653, as previously described (8). The CH#2 vector is driven by the MLV long terminal repeat and is expressed from an internal SV40 promoter. The vector also contains the gene for neomycin resistance.

**Retroviral infection.** The pCH/HER653 plasmid was transfected into PA317 retroviral packaging cells and the supernatant containing the retroviral particles was collected 48 hours after transfection (18). Ovarian cancer cell lines were seeded on a 6-cm dish the day before infection. The cells were then preincubated in polybrene (100 μg/mL) for 1 hour followed by the addition of 1 mL of fresh viral supernatant. They were then plated in medium containing G418 (150 μg/mL) to select for infected cells. After cloning expansion, individual clones were pooled for analysis.

**Anchorage-independent cell growth assays.** To determine their ability for anchorage-independent growth, cells were suspended in 0.3% agar containing media RPMI (Irvine Scientific, Santa Ana, CA) supplemented with 10% fetal bovine serum (FBS; Gemini BioProducts, Calabasas, CA) and penicillin/streptomycin (Irvine Scientific). Five thousand cells mixed with 0.3% of agar were plated onto a base layer of 0.5% agar containing media in 6-well plates. Agar medium (0.3%) was added to cells twice per week. The cell colonies were then counted after 21 days using an inverted light microscope.

**Animals.** Fischer 344 female rats (120-140 g) were obtained from Charles River (Cary, NC) and housed in a pathogen-free animal facility. They were given commercial basal diet and water ad libitum. The Institutional Laboratory Animal Care and Use Committee at the University of California at Irvine, approved the experimental protocol for the use of these animals in the studies.

**Determination of in vivo growth and tumorigenicity.** Trypsin (0.25%) with EDTA (Life Technologies, Gaithersburg, MD) was used to harvest the NuTu-19 cells. These cells were then washed twice with Dulbecco’s PBS solution (Life Technologies) and counted for cell number and viability with trypan blue exclusion. Cell viability of at least 90% was required for experimental use. One million cells in PBS solution were injected i.p. into Fischer 344 rats. The animals were then observed twice daily, weighed weekly, and survival was monitored. All remaining animals were killed just before they died of i.p. carcinomatosis and malignant ascites.

**Selection of in vitro cisplatin-resistant cell line.** The in vitro cisplatin-resistant cell line (NuTu-CPR) was selected by exposing the cells to increasing concentrations of cisplatin (0.1, 0.25, 0.5, and 0.75 μg/mL) over 12 months. Cells were then maintained in cisplatin (0.5 μg/mL; Bristol-Myer Squibb, Princeton, NJ), 3-(4,5-Methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) assays were used to determine the level of cisplatin resistance.

**Selection of in vivo cisplatin-resistant cell line.** Of more clinical relevance, an in vivo cisplatin-resistant cell line (NuTu-ASC) was derived from injecting one million NuTu-19 cells into the i.p. cavity of Fischer 344 rats. After 4 weeks of tumor induction, rats were treated with cisplatin (4 mg/kg) i.p. for four consecutive weeks. Recurrent or persistent tumors were then harvested and plated on monolayer dishes. MTT assays were used to determine the level of cisplatin resistance.

**Cisplatin sensitivity assays.** To assess the cytotoxicity of cisplatin in vitro, 5,000 cells were plated per well in 96-well plates and grown overnight in RPMI supplemented with 10% FBS and antibiotics. With varying concentrations of cisplatin, the cells were incubated for 48 hours. Cell survival was determined by MTT assays (19). Using an ELISA microplate reader (Molecular Devices, Menlo Park, CA), the absorbance was measured at 570 nm. The cisplatin concentration that inhibited cell growth by 50% (IC50) was extrapolated by plotting the drug concentration as a function of percent cell growth inhibition.

**Western blot analysis.** Western blot analyses were used to investigate MAPK activity at the protein level. Parental NuTu-19 and cisplatin-resistant cells (NuTu-CPR and NuTu-ASC) were grown in monolayer culture (80% confluent) at 37°C in a 5% CO2/95% humidified air atmosphere, using RPMI supplemented with 10% FBS and antibiotics. The cells were starved in media with 1% FBS for 12 to 16 hours and then exposed to the EGF ligand (20 ng/mL) for 15 minutes. Crude cell lysates were collected and subjected to SDS/10% PAGE and transferred to nitrocellulose membranes. The blot was incubated with rabbit anti-active MAPK antibodies (Promega, Madison, WI; 1:5,000 dilution) for 1 hour and washed and incubated with a donkey anti-rabbit antibody conjugated with horseradish peroxidase (Promega 1:10,000) for 1 hour. The membranes were subjected to chemiluminescence substrate (Pierce, Rockford, IL) for 5 minutes and exposed to Kodak Biomax ML film.

**Determination of specific binding of 125I-labeled epidermal growth factor to cultured cells.** Subconfluent monolayer cells were washed with 20 mmol/L HEPES-buffered (pH 7.4) HBSS containing 1 mg/mL bovine serum albumin. The cells were then exposed to 1 mL of the same buffer containing 0.05 μg of 125I-labeled EGF and incubated at room temperature for 1 hour. The radioactive medium was then aspirated and the cells were washed with ice-cold buffer. After the cells were dissolved with 2 mL of 0.1 N NaOH containing 1% SDS, they were transferred to glass tubes and the radioactivity was analyzed in an LKB Wallace gamma counter. We calculated specific binding as the total radioactivity bound in the absence of unlabeled hormone minus that bound in the presence of excess unlabeled hormone.

**RNA extraction and Northern blot analysis.** RNA was extracted by acid guanidinium thiocyanate phenol chloroform method (20). The RNA was size-fractionated on 1% agarose/glyoxal/DEMSO gels (Ambion, Austin, TX) and transferred onto nylon membranes and cross-linked by UV irradiation. Blots were prehybridized and hybridized with a cDNA probe and labeled with 32P (3,000 Ci/mm) using random hexanucleotide primers. Under stringent conditions, these blots were washed and exposed at −70°C. cDNA probes were used to hybridize to cytoplasmic RNA. The EGFR cDNA was a HindIII and EcoRI derived fragment (47 bp) from the upstream EGFR-DNR coding region containing the initial AUG codon by restriction digestion.

**Reverse transcription-PCR.** Cellular RNAs were extracted from cultured NuTu cells or their derivatives using the Quiagen RNeasy Protect Mini Kit (Qiagen, Valencia, CA). Two-step reverse transcription-PCR (RT-PCR) reactions were done using Invitrogen SuperScript First-strand Synthesis System for RT-PCR Kit and the Protocol (Invitrogen, Carlsbad, CA). Approximately 5 μg of cellular RNA were used as a template for cDNA synthesis in a final reaction volume of 50 μL. Two microliters of the resulted first strand cDNA were used for PCR amplification. PCR reactions were done in a 96-well Robocycler (Stratagene, La Jolla, CA) and the amplifications were carried out in a volume of 25 μL containing the cDNA, 50 mmol/L KCl, 10 mmol/L Tris-HCl, 2 mmol/L of each deoxynucleotide triphosphate, 20 pmol of each primer, 1.5 mmol/L MgCl2, and 1 unit Taq polymerase (Qiagen), for 5 minutes at 94°C for initial denaturing followed by 25 to 35 cycles of 94°C for 30 seconds, 48°C to 61°C (depending on the primers melting temperatures) for 30 seconds and 72°C for 1 minute, and a final incubation at 72°C for 10 minutes. The following oligonucleotide primers were used for the primers and probes. EGFR forward CGAGACTCCTTCT-TCCTTCTAACCCG, EGFR reverse TGGCGTGTCTCGGGACACCACTAGACGTG, ERRb2 forward GCTCAACAGAGG-ACAGTAAGAACA, ERRb2 reverse GACCCATTGTTCA-GAAC-CTTICA, ERRb3 forward GTTGAAACTACCAACACTTCCAG, ERRb3 reverse GTCCGGTGTATCTACT-CACTC, ERRb4 forward GGGAATCTCATCT-TCTTGFCACT, and ERRb4 reverse GTCCAG-TTGATGGTTGTTAATAA.
RT-PCR products were analyzed by electrophoresis in an agarose gel followed by staining in ethidium bromide and visualization under UV light.

Statistical analysis. Student’s t test was employed for statistical analysis, with P < 0.05 taken as the level of significance. The MTT cell growth assay results were expressed as mean ± SE of three experiments each done in triplicate cultures.

Results

Expression of dominant-negative epidermal growth factor receptor in NuTu-19 cells. Cells were screened by Southern blot analysis for the presence of the truncated EGFR. The cells that expressed EGFR-DNR (NuTu-DNR) exhibited a major band when probed with the radiolabeled EGFR-DNR cDNA fragment (Fig. 1A). By contrast, parental NuTu-19 cells and cells transfected with the empty vector (NuTu-Sham) did not exhibit this band. Northern blot analysis revealed that the cells infected with the truncated receptor expressed the truncated EGFR mRNA transcript (2.5–3 kb), whereas parental NuTu-19 and NuTu-Sham cells did not express this transcript (Fig. 1B). Expression of EGFR-DNR was determined to be stable over time with multiple passages.

Effect of dominant-negative epidermal growth factor receptor on anchorage-independent growth and tumorigenicity. Parental NuTu-19 and NuTu-Sham cells readily grew in an anchorage-independent manner. After 21 days, there were 1,200 ± 250 colonies of NuTu-19 cells following the seeding of 5,000 cells, which translated into a growth efficiency of 24%. Similarly, NuTu-Sham cells formed 1,150 ± 200 colonies. Infection with the EGFR-DNR dramatically inhibited colony formation yielding only 5 ± 2 colonies per soft agar plate, representing a growth efficiency of 0.1% (P < 0.001; Fig. 2). Thus, the expression of EGFR-DNR markedly suppressed anchorage-independent growth in NuTu-19 cells.

Because anchorage-independent growth is often associated with tumorigenicity (21, 22), we next sought to determine the antitumorigenic effect of the EGFR-DNR using an immunocompetent rat model. We found that all rats injected with the NuTu-Sham cells infected with the EGFR-DNR (NuTu-DNR) remained healthy following tumor injection. In contrast, 100% of the rats injected with either parental NuTu-19 or NuTu-Sham cells died of disease progression at the end of 15 weeks (P = 0.00009; Fig. 3). Thus, cells expressing the EGFR-DNR have lost their tumorigenic potential in the immunocompetent rat model.

Effect of dominant-negative epidermal growth factor receptor on mitogen-activated protein kinase pathway. Previous studies have shown that the EGFR-DNR can attenuate downstream signals from EGFR that involve RAS, RAF, MAP/ERK kinase, and MAPK (7, 23). To determine the effect of EGFR-DNR on signaling transduction by the EGF receptor, we evaluated cells that were grown in media with low levels of growth factors. These cells were then briefly treated with EGF for 15 minutes and lysed. Using polyclonal antibodies specifically for phosphorylated MAPK (erk 1 and 2 proteins), both parental NuTu-19 and NuTu-Sham cells showed a strong activation of erk 1 and 2 after exposure to EGF. By contrast, when EGF was added to the NuTu-DNR cells, there was a marked attenuation in erk 1 and 2 phosphorylation (Fig. 4A). Thus, EGF blockade altered the signal transduction pathway by reducing phosphorylated MAPK activity in NuTu-DNR cells.

Effect of dominant-negative epidermal growth factor receptor on cisplatin resistance. The mechanisms that determine ovarian cancer cell sensitivity to cisplatin are not well established. Previous research on human ovarian cancer cell lines have shown that elements of the signal transduction pathway activated by EGF determine the sensitivity of ovarian cancer cells to cisplatin, and that a cisplatin-resistant phenotype was associated with an alteration in this signal transduction pathway (13, 14, 24). Thus, selection of cisplatin resistance affected the response of ovarian cancer cells to the EGF ligand. We were interested in determining the relationship of cisplatin resistance and EGF response in the NuTu-19 system. We selected cells that were resistant to cisplatin both in vitro and in vivo. The level of resistance in NuTu-19, NuTu-CPR (in vitro selection), and NuTu-ASC (in vivo selection) were analyzed with MTT assays. The NuTu-CPR cells were 10 times more resistant than the parental NuTu-19 cells, as quantified by the IC50 values. The NuTu-ASC cells were 3.2 times more resistant compared with the parental NuTu-19 cells. Based on an analysis of the resistance curves, it seemed that there is more than one population of resistant cells with differing sensitivities within the NuTu-CPR and NuTu-ASC resistant cell lines.
lines. For example, there is a subpopulation of resistant cells in the NuTu-CPR cell line, accounting for ~30% of the cells that were substantially more resistant (Fig. 6A). Cells were then maintained in cisplatin (0.75 μg/mL). However, once acquired, the resistance was stable for over 2 years even without cisplatin maintenance.

Western blotting revealed that the cisplatin-resistant cells, NuTu-CPR and NuTu-ASC, showed an enhanced EGF stimulatory effect via the MAPK pathway compared with parental cells (Fig. 4B). However, expression of EGFR-DNR attenuated the EGF-mediated stimulation of MAPK activity in both types of resistant cells (Fig. 4C and D).

Specific binding of radiolabeled epidermal growth factor and expression of epidermal growth factor receptor RNAs in cell lines. In theory, it is possible that the difference in the EGF stimulation of MAPK phosphorylation within the cisplatin-sensitive and cisplatin-resistant cells was related to increases in cell surface EGF binding as opposed to alterations in signal transduction. However, parental NuTu-19 cell lines bound radiolabeled EGF more readily than NuTu-CPR and NuTu-ASC cells. Furthermore, vectored expression of the DNR did not change the ability of these cells to bind radiolabeled EGF (Fig. 5A). Thus, the DNR effects on the growth and signaling properties of the cisplatin-resistant cells were not due to changes in EGF binding or receptor up-regulation.

Another possible explanation for the increased MAPK signaling from EGF in the cisplatin-resistant cells could be that the resistant cells expressed higher levels of other EGFR receptors, including erbB2, erbB3, and erbB4. These receptors can heterodimerize with EGFR and thus transmit signals from EGF. To test this, total RNA from NuTu-19, NuTu-CPR, and NuTu-ASC cells were analyzed by quantitative RT-PCR to determine whether they showed increased expression of erbB2, erbB3, or erbB4 RNAs (see Fig. 5B). Whereas erbB2 and erbB3 RNAs could be detected, we did not find an overexpression in the chemoresistant cell lines. In addition, erbB4 RNA was not detected in either the parental or cisplatin-resistant cell lines. Thus, altered expression of other EGFR family members was not likely responsible for the enhanced MAPK activation in the cisplatin-resistant cells.

Reversal of cisplatin resistance. NuTu-CPR and NuTu-ASC cells showed a strong activation of MAPK by EGF that could be attenuated by the EGFR-DNR. We next sought to determine whether inhibition of EGFR signaling might reverse the cisplatin resistance in these cells. NuTu-CPR cells expressing EGFR-DNR were approximately twice more sensitive to the cytotoxic effects of cisplatin compared with NuTu-CPR cells (Fig. 6B). Similarly, NuTu-ASC cells expressing EGFR-DNR were four times more sensitive to the cytotoxic effects of cisplatin compared with NuTu-ASC cells (Fig. 6C). Thus, the EGFR-DNR has the ability to partially reverse the cisplatin resistance in both in vitro (NuTu-CPR) and in vivo (NuTu-ASC) selected cisplatin-resistant cell lines.

Discussion

Despite many recent advances in chemotherapeutic agents, the 5-year survival rate of women diagnosed with advanced-stage epithelial ovarian carcinoma is only 20% (2). Previous studies have established that epithelial ovarian cancers, as well as other poor prognostic malignancies, overexpress EGFR and other members of the EGFR family (3, 4, 25). Clinical studies have shown that elevated EGFR levels in tumor cells are associated with decreased survival in patients (26, 27). These reports suggest that the EGFR may confer a growth advantage to ovarian cancer cells. Moreover, the EGF pathway and its autocrine mechanism may be important for tumor cell growth.

![Figure 3](image3.png)  
*Figure 3.* In vivo studies in an immunocompetent rat model. NuTu-19, NuTu-Sham and NuTu-DNR cells were injected i.p. into groups of five Fischer 344 rats with one million cells. Animals were observed twice daily and weighed weekly and survival was monitored. (P = 0.00009).

![Figure 4](image4.png)  
*Figure 4.* A-D, effect of EGFR-DNR and cisplatin resistance on NuTu-19 cell signal transduction. NuTu-19, NuTu-Sham, and NuTu-DNR (A) were incubated for 15 minutes in the presence (+) and absence (−) of EGF (20 ng/mL). Cell lysates were collected and subjected to SDS/10% PAGE and transferred to nitrocellulose membranes. The membranes were probed with anti-active MAP kinase antibodies. Visualization was by chemiluminescence. Total MAPK antibody was used to ensure equal loading of protein samples. NuTu-19, NuTu-CPR, and NuTu-ASC (B); NuTu-CPR, NuTu-CPR-Sham, and NuTu-CPR-DNR (C); NuTu-ASC, NuTu-ASC-Sham, and NuTu-ASC-DNR cells (D) were subjected to the experiment as previously described in (A).
There are various challenges in developing novel therapeutic targets in the treatment of epithelial ovarian cancer. For example, it is difficult to develop a reliable and reproducible animal model analogous to human epithelial ovarian cancer. The immunocompetent rat model employed in this study is applicable because it resembles the clinical and biological behavior of human ovarian cancer. The NuTu-19 cell line was derived from epithelial cells and is described as a poorly differentiated papillary serous ovarian adenocarcinoma based on histologic analysis (28). The process of malignant transformation was achieved spontaneously by the repetitious growth of rat ovarian surface epithelial cells in vitro, which has been hypothesized to contribute to the initiation of ovarian carcinogenesis in humans (28). Although the human tumor nude mouse xenograft has shown utility in ovarian cancer research, this model lacks a competent immune system. Thus, studies that require the analysis of the immune response to both the implanted cancer cells and experimental therapy would necessitate an animal model with an intact immune system.

The NuTu-19 ovarian cancer rat model can address issues such as the behavior of early disease and response to experimental chemotherapy and immunotherapy. As is typical of clinical human ovarian cancer, this model shows low immunogenicity in the presence of a syngeneic intact immune system. Furthermore, the NuTu-19 cells express low levels of MHC class II antigens that allow the tumor cells to escape immune recognition (28). In this report, we employed the NuTu-19 ovarian cancer rat model to determine whether abrogation of EGFR-dependent signaling pathways by a EGFR-DNR blockade alters the growth and chemoresistance of NuTu-19 cells. Expression of EGFR-DNR by retroviral infection attenuated the effects of EGF on EGFR tyrosine phosphorylation, and inhibited growth in several clones. These observations further substantiate the hypothesis that ovarian cancer cell growth is partially EGFR dependent and underscore the potential therapeutic role of EGFR-DNR in ovarian cancer.

EGFR is a member of the type I tyrosine kinase growth factor receptor family and has structural similarities with HER-2, HER-3, and HER-4 receptors. Given the structural homology of these receptors, the EGFR-DNR is capable of heterodimerizing with EGFR and other members of the EGFR family such as HER-2, HER-3, and HER-4 (11). Furthermore, because many of these receptors are coexpressed in ovarian carcinomas, any tumors

Figure 5. A, effect of time on the binding of 125I-labeled EGF to NuTu-19 cells. NuTu-19, NuTu-CPR, and NuTu-ASC cells were incubated with 0.05 ng of 125I-labeled EGF at room temperature for over four hours. The cells were then lysed with 0.1 N NaOH and specific binding was determined. B, RT-PCR for EGFR family RNAs. RNAs from NuTu-19 or the cisplatin-resistant cells were analyzed for the presence of EGFR family RNAs by RT-PCR. For each oligonucleotide primer set, the same amount of RNA and same number of PCR cycles was used for all of the RNAs. Agarose gel electrophoresis of equal samples from the reactions is shown. The results are in the linear range, because the same relative intensities of the product bands were observed when fewer amplification cycles were employed.

Figure 6. A, sensitivity to cisplatin. NuTu-19, NuTu-CPR, and NuTu-ASC were incubated in the presence of indicated concentrations of cisplatin. MTT assays were then performed as described in the Materials and Methods. Points, mean % change from unstimulated controls of eight determinations per experiment from three separate experiments; bars, ± SE. P < 0.05 when compared with respective controls. B, effect of EGFR-DNR on in vitro selected cisplatin-resistant cells. NuTu-CPR and NuTu-CPR-DNR were subjected to the experiment described in (A). P < 0.05 when compared with respective controls. C, effect of EGFR-DNR on in vivo selected cisplatin-resistant cells. NuTu-ASC and NuTu-ASC-DNR were subjected to the experiment described in (A). P < 0.05 when compared with respective controls.
overexpressing any member of the EGFR family may be susceptible to the inhibitory effects of the truncated receptor. This increases the potential usefulness of the truncated EGFR as a means for antitumor therapy. As the most active agent in the treatment of ovarian cancer, cisplatin inhibits the growth of rapidly dividing cells by developing same-strand DNA cross-links and inducing apoptosis (24, 29). Cancer cell resistance to chemotherapy involves many factors, including cellular drug transport, intracellular drug metabolism, imbalance of apoptotic factors, and aberrant cell cycle regulation (30). Altered growth factor signaling pathways may also attenuate cellular responses to chemotherapy (24). In this report, we found that cisplatin-resistant cell lines were dependent on the MAPK pathway for growth, as evidenced by the attenuation of EGF-mediated MAPK activity with the EGFR-DNR. Furthermore, inhibition of EGFR signaling sensitized these chemoresistant cells to the effects of cisplatin. Thus, the modulation of EGFR signaling may enhance cisplatin-induced cell killing in resistant ovarian cancers.

The cisplatin-resistant cells in our NuTu-19 cell model exhibited an enhanced MAPK activation compared with chemoresistant cells without an increase in radiolabeled EGF binding. Whereas some studies have shown that drug-resistant ovarian and cervical cancers exhibited a lower level of EGFR expression (31, 32), other reports have shown that multidrug resistance was associated with enhanced EGFR expression (33). Moreover, studies have revealed that expression of a truncated EGFR can suppress EGF-induced mitogenic response of ovarian cancer cells, as well as the tumorigenic activity of transformed mouse fibroblasts (34, 35). Our previous study showed that EGFR-DNR expression was associated with an inhibition of pancreatic cancer cell growth and enhanced sensitivity to cisplatin (8). This current report confirmed the antitumorigenic activity of the truncated EGFR using a rat ovarian cancer cell line. Furthermore, these studies showed that the truncated EGFR suppressed ovarian cancer cell tumorigenicity and evaded cisplatin resistance in an immunocompetent rat model. Together, these findings suggest that the EGFR-DNR strategy may ultimately prove to be a useful therapeutic tool in the treatment of cisplatin-sensitive and cisplatin-resistant ovarian cancers.

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References

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