Discovery of Differentially Expressed Genes Associated with Paclitaxel Resistance Using cDNA Array Technology: Analysis of Interleukin (IL) 6, IL-8, and Monocyte Chemotactic Protein 1 in the Paclitaxel-resistant Phenotype

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ABSTRACT
In an attempt to define the molecular changes associated with the paclitaxel-resistant phenotype in human cancer, a paclitaxel-resistant ovarian cancer cell line, SKOV-3TR, was established through stepwise selection in increasing paclitaxel concentrations. SKOV-3TR was cross-resistant to doxorubicin and vincristine and overexpressed multidrug resistance gene 1 but not multidrug resistance associated protein. SKOV-3TR and the paclitaxel-sensitive SKOV-3 parent line were characterized using human cDNA array technology that examined expression of a wide variety of genes involved in cell growth, signal transduction, cell death, and immune function. cDNA probes from reverse transcribed mRNAs of both paclitaxel-resistant and parent cells were compared to identify genes differentially expressed in the paclitaxel-resistant cells. Of 588 different human cDNA transcripts compared, 6 genes were found to be markedly decreased, and 12 genes increased in the resistant subline. Northern analysis and/or reverse transcription-PCR confirmed that 12 of these 18 genes were over- or underexpressed in SKOV-3TR. In addition, at least eight of the genes were found differentially expressed in several other paclitaxel- and/or doxorubicin-resistant cell lines, both those with increased multidrug resistance expression and those without. Included in the set of overexpressed genes were the cytokines/chemokines interleukin 6, interleukin 8, and monocyte chemotactic protein 1. ELISA assays confirm that mRNA overexpression of these cytokine/chemokines was associated with the increased secretion of these molecules in the tissue culture supernant. Evaluation of supernatants from an expanded collection of paclitaxel- and Adriamycin-resistant cell lines demonstrated that all of the resistant lines had significant overexpression of at least one cytokine/chemokine as compared with their drug-sensitive parent line. The overexpression of these cytokines seemed to be stable and associated with a drug-resistant phenotype with only a modest induction of cytokine expression in the parent line with short-term paclitaxel exposure. These findings suggest that the development of paclitaxel resistance is accompanied by multiple changes in gene expression including stable alterations in selective chemokine and cytokine expression. The role these associated genetic changes have in the drug-resistant phenotype is discussed.

INTRODUCTION
Paclitaxel is a novel anticancer agent with activity in a broad range of epithelial cancers, including carcinomas of ovary, breast, head, neck, bladder, lung, and prostate (1–4). The efficacy of paclitaxel is limited by the development of drug resistance in a population of surviving malignant cells. Defined molecular mechanisms for acquired tumor cell resistance to paclitaxel include overexpression of the drug efflux pump MDR1 and differential expression of β-tubulin isotypes or β-tubulin gene point mutations (5–8). Recent data also suggest that p53 status and mitosis checkpoint control is important in determining the sensitivity of cells to paclitaxel (9, 10). In general, there is little ex vivo clinical data and limited understanding of which, if any, of these mechanisms influences paclitaxel resistance in humans. Whereas most drug resistance studies have focused on a few specific gene(s), the aim of this study was to define broader patterns of change by evaluating

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3 The abbreviations used are: MDR1, multidrug resistance gene 1; ADR, Adriamycin; CIP2, protein tyrosine phosphatase; GADD45, growth arrest and DNA-damage-inducible protein gene; IL, interleukin; MCP-1, monocyte chemotactic protein 1; MIP2α, macrophage inflammatory protein-2-α; MRP, multidrug resistance-associated protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NKEFβ, natural killer cell enhancing factor B; RIZ, retinoblastoma interacting zinc finger gene; RT-PCR, reverse transcription-PCR; VEGF, vascular endothelial growth factor; ERBB-2, receptor protein tyrosine kinase.

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differential gene expression between paclitaxel-sensitive and -resistant cells. Techniques commonly used to determine gene expression profiles include RT-PCR, Northern blot analysis, and RNase protection. However, these methods of necessity examine only one gene at a time. A more promising approach, cDNA array analysis, has recently developed and allows rapid evaluation of large numbers of expressed genes. This technique, involving the hybridization of cellular cDNA to high-density nucleic acid arrays, is well suited for evaluating large numbers of gene transcripts. (11–13).

To evaluate the patterns of gene expression in paclitaxel-sensitive and -resistant ovarian cancer cells, we established a paclitaxel-resistant human ovarian cancer cell line, SKOV-3 TR, from its paclitaxel-sensitive parent, SKOV-3, and compared mRNA levels in the two lines using cDNA array technology. Genes demonstrating changed expression were selected for further evaluation in several other paclitaxel- and/or doxorubicin-resistant cell lines, with particular emphasis on a collection of chemokine and cytokine genes overexpressed in the SKOV-3 TR cell line.

**MATERIALS AND METHODS**

**Cell Culture, Selection, Drugs, and Cytotoxicity Assays.** Human ovarian cancer cell line SKOV-3 and some of the other cancer cell lines used in this study were obtained from the American Type Culture Collection (Rockville, MD). Human multiple myeloma doxorubicin-resistant cell line 8226/DOX40 (maintained in the presence of 2.8 μM doxorubicin) and its sensitive counterpart 8226/S were kindly provided by Dr. William S. Dalton (University of South Florida, H. Lee Moffitt Cancer Center, Tampa, FL; Ref. 14). Human breast tumor ADR-resistant cell line MCF-7/ADR (maintained in the presence of 1.7 μM ADR) and its parent cell line, MCF-7, were provided by Dr. Jack Chen (University of Texas, M. D. Anderson Cancer Center, Houston, TX). β-tubulin mutant ovarian carcinoma cell lines PTX 10 and PTX 22 and their sensitive parental line 1A9 were kindly provided by Dr. Tito Fojo (NIH, Bethesda, MD) (8). SKOV-3 and SKOV-3 TR were grown in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin and streptomycin at 100 units/ml and 100 μg/ml, respectively (Life Technologies, Inc., Grand Island, NY). Cells were incubated at 37°C in 5% CO2-95% air atmosphere and passaged twice every 6 days. All of the drugs were obtained from commercial sources. SKOV-3 TR was selected from SKOV-3 by stepwise increases in paclitaxel concentrations from 0.0001 μM to 0.3 μM.

In vitro cytotoxicity was measured using a tetrazolium-based semiautomated colorimetric (MTT) assay, as described previously (15). The absorbance values were normalized assigning the value of the parent line in media to 1.0 and the value of the no cell blank to 0. Experiments were performed in duplicate.
Preparation and Labeling of Gene Probes. Poly(A)⁺ RNA was prepared from SKOV-3 and SKOV-3TR cells using FastTrack mRNA isolation kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Contaminating DNA in the samples was removed by treatment with RNase-free DNase (Boehringer Mannheim, Indianapolis, IN). Probes were synthesized from SKOV-3 and SKOV-3TR mRNA and reagents (CLONTECH) following the manufacturer’s protocol. [³²P]dATP (3.5 μCi/ml) Amersham] was used to label each cDNA probe.

Column Chromatographic Purification of Complex Probes. After reverse transcription, cDNA probes were purified using CHROMA SPIN-200 DEPC-H₂O column (CLONTECH). The labeled cDNAs were applied to the center of the gel-bed’s flat surface and allowed to absorb into the resin (CLONTECH). The labeled cDNAs were applied to the center of the gel-bed’s flat surface and allowed to absorb into the resin (CLONTECH). The labeled cDNAs were applied to the center of the gel-bed’s flat surface and allowed to absorb into the resin (CLONTECH). The labeled cDNAs were applied to the center of the gel-bed’s flat surface and allowed to absorb into the resin (CLONTECH).

Atlas Human cDNA Expression Array and Hybridization. cDNA arrays (CLONTECH) contain 200–500-bp gene fragments that lack poly(A) tails, repetitive elements, or other highly homologous sequences. The gene fragments cover several groups of genes important in cell growth, differentiation, activation and death. Plasmid and bacteriophage DNAs are included as negative controls to confirm hybridization specificity, along with nine housekeeping cDNAs as positive controls for normalizing mRNA abundance. Two identical cDNA array membranes were hybridized individually with freshly labeled and purified cDNA complex probes made from SKOV-3 and SKOV-3TR mRNAs. The arrays were prehybridized using ExpressHyb solution (CLONTECH) for 30 min at 68°C (16) with continuous agitation and were hybridized overnight at the same conditions. Arrays were washed for 20 min at 68°C with 2 × SSC-1% SDS four times, then for 20 min at 68°C with 0.1 × SSC-0.5% SDS two times. The damp membranes were immediately wrapped in plastic wrap, and they were exposed to Kodak BioMax MS X-ray film with an intensifying screen at −70°C.

Table 1 Drug-resistant phenotypes of paclitaxel- and doxorubicin-resistant cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MDR⁺</th>
<th>MRP⁻</th>
<th>LRP⁺⁺</th>
<th>In vitro exposure</th>
<th>Drug IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKOV-3</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻³ × 3.5</td>
</tr>
<tr>
<td>SKOV-3TR</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻¹ × 3.5</td>
</tr>
<tr>
<td>NIH OVCAR</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻⁴ × 5.0</td>
</tr>
<tr>
<td>NIH OVCAR</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻³ × 1.7</td>
</tr>
<tr>
<td>SW626</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻³ × 1.2</td>
</tr>
<tr>
<td>SW626TR</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻³ × 6.0</td>
</tr>
<tr>
<td>CAOV3</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻⁴ × 6.5</td>
</tr>
<tr>
<td>CAOV3TR</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻⁴ × 4.5</td>
</tr>
<tr>
<td>MDA 435</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻⁴ × 4.5</td>
</tr>
<tr>
<td>MDA 435TR</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻⁴ × 4.5</td>
</tr>
<tr>
<td>MCF-7</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻⁴ × 1.1</td>
</tr>
<tr>
<td>MCF-7TR</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻⁴ × 1.8</td>
</tr>
<tr>
<td>I.A9</td>
<td>–</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻³ × 6.0</td>
</tr>
<tr>
<td>PTX10</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–/+</td>
<td>10⁻³ × 2.0</td>
</tr>
<tr>
<td>PTX22</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–/+</td>
<td>10⁻³ × 2.0</td>
</tr>
<tr>
<td>8226/S</td>
<td>–</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻³ × 1.2</td>
</tr>
<tr>
<td>8226/DOX40</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>10⁻³ × 1.2</td>
</tr>
<tr>
<td>8226/MDR10</td>
<td>+</td>
<td>–/+</td>
<td>+</td>
<td>–/+</td>
<td>10⁻³ × 1.1</td>
</tr>
</tbody>
</table>

Expression was measured using Northern analysis.
LRF, lung-resistance-related protein; –, signals were absent; –/+ , signals were weakly positive; +, signals were significantly positive; ND, no data; IC₅₀, drug concentration at which cell growth is inhibited by 50%.

RESULTS

Paclitaxel-resistant SKOV-3 Cell Line, SKOV-3TR-
SKOV-3TR was selected from its parent cell line by exposure to stepwise increases in paclitaxel concentrations (from 0.0001 μM
The resistant phenotype was found to be stable after 18 months of continuous culture in 0.3 μM paclitaxel. MTT cytotoxicity analysis shows that SKOV-3 TR cells are 100-fold more resistant to paclitaxel as compared with the sensitive parent cell line (IC50s, 350 nM versus 3.5 nM, respectively). In addition, SKOV-3 TR also demonstrates resistance to vincristine and partial cross-resistance to doxorubicin (Fig. 1). Northern analysis reveals strong expression of MDR1 in SKOV-3 TR as compared with no detectable MDR1 expression in SKOV-3. There is no change in MRP expression, whereas lung-resistance-related protein shows a modest 2-fold increase in expression in SKOV-3 TR (Fig. 2; Table 1).

**Genes Associated with Paclitaxel Resistance.** Human cDNA arrays were used to examine relative gene expression between SKOV-3 and SKOV-3 TR. Approximately 15% of the 588 genes were expressed at a level of visual detection on the cDNA arrays. Comparative expression analysis of SKOV-3 versus SKOV-3 TR in the two experiments revealed 18 genes displaying altered signal intensity (Fig. 3). Of the 18 differentially expressed genes, 6 were decreased in expression in SKOV-3 TR, whereas 12 showed increased expression in SKOV-3 TR including several cytokine and chemokine genes, namely IL-6, IL-8, MIP2α, and MCP-1 (Table 2).

**Northern Blot and/or RT-PCR Analysis of Differentially Expressed Genes.** To confirm array results, gene expression was measured by Northern analysis and/or RT-PCR. Differential expression of 12 of the 18 genes was confirmed with >3-fold differences as determined by Northern analysis (Fig. 4) and/or RT-PCR in all cases. cDNA probes of 6 of the 18 genes were not confirmed. Five of the six genes demonstrated less than 2-fold difference in expression by Northern analysis, whereas cyclin A showed no difference in RNA expression by RT-PCR (Table 2).

**Analysis of Differentially Expressed Genes in Other Drug-resistant Cells.** Differential expression of these 12 genes in the drug-resistant phenotype was studied in several other lines, selected for resistance to paclitaxel or doxorubicin. After 3–6 months of continuous culture in paclitaxel, breast and ovarian cancer cell lines were 10- to 70-fold resistant to paclitaxel as compared with their parent lines. Northern analysis revealed overexpression of MDR1 in all of the paclitaxel-resistant cell lines as compared with their parental cells. MRP expression was not altered between resistant and sensitive cell lines (Fig. 2; Table 1). Northern analysis and RT-PCR evaluating expression of genes identified in the cDNA array analysis in these cell lines have again shown an association of these genes with the drug-resistant phenotype.

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**Table 2: Comparison of gene expression with cDNA array to Northern analysis and/or RT-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>cDNA array</th>
<th>Northern/and or RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP2α</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>MCP-1</td>
<td>↑</td>
<td>↑*</td>
</tr>
<tr>
<td>IL-8</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>CIP2</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>GADD45</td>
<td>↑</td>
<td>↑*</td>
</tr>
<tr>
<td>Transforming growth factor-β2</td>
<td>↑</td>
<td>↑*</td>
</tr>
<tr>
<td>Helix-loop-helix protein 1R21</td>
<td>↑</td>
<td>↑*</td>
</tr>
<tr>
<td>VEGF</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>ERBB-2</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>NKEFB</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Amxin 1 (RIZ)</td>
<td>↓*</td>
<td></td>
</tr>
<tr>
<td>Fos-related antigen 1 (FRA-1)</td>
<td>↓</td>
<td>NC</td>
</tr>
<tr>
<td>Nucleobindin</td>
<td>↓</td>
<td>NC</td>
</tr>
<tr>
<td>Cyclin B1</td>
<td>↑</td>
<td>NC</td>
</tr>
<tr>
<td>Cyclin A1</td>
<td>↑</td>
<td>NC*</td>
</tr>
<tr>
<td>Chaperonin</td>
<td>↑</td>
<td>NC</td>
</tr>
<tr>
<td>DNA topoisomerase Ha Isocyn</td>
<td>↑</td>
<td>NC</td>
</tr>
</tbody>
</table>

*Upward arrows indicate higher signals in SKOV-3 TR than in SKOV-3. Downward arrows indicate lower signals in SKOV-3 TR. Double arrows indicate cDNA array results confirmed by Northern analysis and/or RT-PCR. Double arrows or “NC” followed by asterisks were evaluated by RT-PCR with all other evaluations by Northern analysis. “NC” nonconfirmatory, indicates the altered expression of a particular gene could not be validated or was inconsistent by Northern analysis or RT-PCR.
tively overexpressed in the Taxol-resistant cell lines SW626_TR, CAOV-3_TR, and MDA435_TR, as compared with their paclitaxel-sensitive parental lines, and in the human multiple myeloma doxorubicin-resistant cell lines 8226/DOX 40 and 8226/MDR 10 V (Fig. 5; Table 3). Interestingly, β-tubulin mutant ovarian carcinoma cell lines PTX10 and PTX22 overexpress the CIP2 mRNA but do not show differences in expression of the other genes identified in this study. Of note, MCF-7_TR does not differentially express any of the 12 genes whose expression is altered in the paclitaxel-resistant phenotype, SKOV-3_TR.

**Increased IL-6, IL-8, and MCP-1 Expression in Paclitaxel/ADR-resistant Cell Lines.** Subsequent analysis focused on a subset of the chemokine and cytokine genes identified through this study. Particular attention was given to the kinetics of gene induction and the verification that changes in gene expression were associated with protein expression. IL-6, IL-8 and MCP-1 concentrations in culture supernatants of resistant and sensitive cell lines were examined by ELISA. SKOV-3_TR, SW626_TR, CAOV-3_TR, NIH:OVCAR-3_TR, and MCF-7/ADR produced high levels of IL-6 (Table 4). In contrast, secretion of IL-6 by MCF-7_TR, MDA 435_TR, and 8226/DOX 40 cell lines were nearly equal to the secretion of the cytokine by the sensitive parental cell lines. IL-8 and MCP-1 were overexpressed in all of the paclitaxel- and ADR-resistant cell lines, but the magnitude of overproduction on culture varied significantly between lines (Table 4). Analysis of this data demonstrated that the cytokine values were not normally distributed, and, hence, a Wilcoxon sum-rank test was performed. All three of the cytokine/chemokines were significantly elevated in the drug-resistant cell lines as compared with the drug-sensitive parental lines (IL-6 and IL-8: both, \( P < 0.04 \); MCP-1, \( P = 0.009 \)).

**IL-6, IL-8, and MCP-1 Induction in Parental Cells.** To determine whether the induction of cytokine expression was a result of rapid induction by paclitaxel prior to the establishment of the drug-resistant phenotype, IL-6, IL-8, and MCP-1 concentrations were evaluated after the paclitaxel-sensitive parent cell lines were exposed to paclitaxel for 48 h. After exposure, there were only modest increases of cytokine expression, and less than that was seen in cells with a paclitaxel-resistant phenotype (Fig. 6).

To evaluate whether the overproduction of these cytokines in the chemotherapy-resistant cells is dependent on continuous drug exposure, the resistant cell lines were grown both in and out of either paclitaxel or ADR for 1 week. At this time, the supernatants were collected and an analysis of the cytokines was performed. Supernatants from resistant cell lines had nearly equivalent levels of IL-6, IL-8, and MCP-1 with or without concurrent paclitaxel/ADR exposure, which suggests that the drug-resistant cell lines constitutively secrete cytokines independent of drug exposure (data not shown).
Kinetics of IL-6 Production. To exclude the possibility that different cytokine concentrations represented differences in cell densities, the kinetics of changes in IL-6 concentration were evaluated in both parental and drug-resistant SKOV-3 plated at $1 \times 10^5$ cells in fresh media with supernatants collected at different time points for IL-6 measurements. The data indicate IL-6 levels accumulated rapidly in SKOV-3TR both in and out of paclitaxel at levels markedly above that of the SKOV-3 parental line. This secretion continued to increase over time up to 96 h (Fig. 7). MTT-evaluated cell growth of the SKOV-3 and SKOV-3TR cell lines demonstrates nearly equivalent growth rates with the SKOV-3 cell line demonstrating slightly greater growth rates as compared with the drug-resistant daughter cell line (data not shown).

DISCUSSION

This study uses high density cDNA array technology to demonstrate the transcriptional differences between the paclitaxel-sensitive SKOV-3 parental line and the paclitaxel-resistant daughter line, SKOV-3TR. The results reveal that 18 of the 588 transcripts screened have clearly discernible changes between the two cell lines. Northern and RT-PCR techniques confirm transcriptional changes in 12 of the 18 candidate genes. These findings suggest that cDNA array analysis offers the advantage of a low false-positive rate when compared with differential display technology. The latter technique is also labor-intensive and time-consuming (17–19).

Our analysis was confined to a small portion of the total cellular transcripts and, hence, does not represent a complete transcriptional profile of the SKOV-3TR drug-resistant phenotype. Review of the 12 genes with altered expression identifies 2 genes, previously linked to the drug-resistant phenotype, ERBB-2 and VEGF (20, 21). Review of the 10 remaining genes reveals that 5 are chemokine or cytokine genes whereas the other 5 genes—CIP2 (22), RIZ (23), NKEFB (24), GADD45 (25), and fos-related antigen 1 (FRA-1) (26) have not been previously linked to drug resistance.

Changes in chemokine/cytokine gene expression were observed for IL-6, IL-8, MCP-1, and MIP-1α, but not for IL-1 through -5, IL-7 IL-9, IL-10 through -13, IL-15, IL-17, IFN-γ, granulocyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, or macrophage-colony stimulating factor. These results are the first to demonstrate the association of MCP-1 secretion with the paclitaxel (and ADR) multidrug-resistant phenotype and add further evidence to the association of IL-6 and IL-8 secretion with clinical paclitaxel resistance. This association has been established in the past for both breast and ovarian carcinoma cell lines as well as a multiple myeloma cell line. The overproduction of these cytokines seems not to represent direct induction by drugs but rather to...
reflect constitutive production from cells expressing a stable multidrug-resistant phenotype even when cells are grown in the absence of paclitaxel. Essentially all of the drug-resistant cell lines overexpress IL-8 and MCP-1 chemokines with a subset also overexpressing IL-6, which suggests that these proteins may serve as important survival factors that promote the multidrug-resistant phenotype.

IL-6 is a multifunctional cytokine that has been shown to play an important role in immune host-defense function, hematopoiesis, and hepatocyte growth (27, 28). Other studies suggest a role for these cytokines in normal epithelial cell growth, including ovarian epithelium (29). IL-6 is also a growth factor for several tumors, most notably myeloma but also renal cell and cervical carcinomas (30–32). Also, elevated levels of IL-6 have been found in the serum or ascites of patients with different tumors. These levels are associated with poor prognosis in women with ovarian and breast carcinoma (33, 34) as well as in persons with other malignancies (35, 36). IL-6 is an inhibitor of cytotoxic chemotherapy-associated apoptosis in in vitro systems. For example, IL-6 secretion protects the hormone-resistant prostate tumor cells against the cytotoxic effects of cisplatin and etoposide and its neutralization sensitizes the cells to cytotoxicity (37, 38). It is possible that elevated levels of IL-6 in the tumor microenvironment lead to local chemotherapy resistance. Curiously, paclitaxel resistance has been associated with changes in proapoptotic molecules such as Bax as well as the anti-apoptotic molecule BCL-2 (39, 40).

IL-8 has been shown to enhance angiogenesis and tumor metastasis (41, 42). Study of IL-8 demonstrates that this cytokine is overexpressed in malignant as compared with benign ovarian cysts (43). Increased IL-8 expression has also been linked to aggressive cases of ovarian cancer in preclinical models and women with ovarian cancer (44–46). Other investigators have demonstrated that short-term exposure of paclitaxel to ovarian cancer cell lines in vitro caused the induction of IL-8, but not that of IL-6, in a subset of ovarian cancer cell lines after 18 h of paclitaxel exposure (47). Our results demonstrate modest induction of IL-8 by paclitaxel in a subset of both human breast and ovarian tumor cells, although the levels of cytokine overexpression are much higher than those in the short-term induction experiments. These data suggest that IL-8 may play a more general role in the multidrug resistance phenotype or the stress response of cells exposed to an antineoplastic agent.

MCP-1 is a low molecular weight chemotactic chemokine important in the migration of monocytic cells as well as acute and chronic inflammatory processes (48). Immunohistochemi-
...studies by Negus et al. (49, 50) have demonstrated that ovarian carcinoma cells express MCP-1 in tumor blocks. These tumors characteristically have immune cell infiltrates, presumably recruited by MCP-1, and other chemokines or cytokines expressed by the tumor cells or surrounding stroma.

There exists the possibility that some of the differentially expressed genes represent epigenetic and/or stress responses to paclitaxel and, thus, are more accurately considered paclitaxel resistance-associated genes rather than genes directly responsible for the paclitaxel-resistant phenotype. Further evaluation of this hypothesis requires functional studies of the individual candidate genes in transfection experiments.

In summary, cDNA array technology provides a rapid and powerful technique for continuing to define the constellation of genes involved in paclitaxel resistance. It is conceivable that the sum total of genes involved in the paclitaxel-resistant phenotype within a single cell line is very large. Whereas many of these changes may be epigenetic, it is possible that unigenetic mechanisms of drug resistance are the exception rather than the rule. This is not to suggest that the expanding pool of ATP-binding cassette transporter pumps (MDR1 and the MRP family) are not important in clinical drug resistance, but rather that the cellular response to paclitaxel may be influenced by a spectrum of genes that control proliferation, checkpoint function, and apoptosis. This hypothesis, if confirmed by functional studies, will have broad implications related to the efforts currently underway to reverse multidrug resistance in the clinical setting.

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