Autocrine Production of Interleukin 6 Causes Multidrug Resistance in Breast Cancer Cells

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ABSTRACT

It has been shown that serum levels of interleukin (IL)-6 are elevated in patients with various types of cancer. However, the exact source of IL-6 in these patients and its role in tumor progression remain unclear. Here we demonstrate that the autocrine production of IL-6 by tumor cells promotes resistance of the cells to chemotherapy, a novel function of IL-6 in cancer biology. Breast cancer cells that are sensitive to drug treatment do not express IL-6, whereas high levels of IL-6 are produced by multidrug-resistant breast cancer cells. Expression of the IL-6 gene in drug-sensitive breast cancer cells increases their resistance to drug treatment by activating the CCAAT enhancer-binding protein family of transcription factors and inducing mdrl gene expression. Thus, the autocrine production of IL-6 by tumor cells is an important factor in determining the susceptibility or resistance of these cells to drug treatment. Because tumors from some breast cancer patients contain IL-6-producing cells, it is possible that IL-6 could potentially be used as a prognostic factor for chemotherapy resistance.

INTRODUCTION

IL-6 is a cytokine produced by a number of cell types including fibroblasts, macrophages, T and B lymphocytes, endothelial cells, glial cells, and keratinocytes in response to a variety of external stimuli (e.g., IL-1, tumor necrosis factor, and platelet-derived growth factor). IL-6 induces synthesis of acute phase response proteins in hepatocytes, terminal differentiation of B cells to antibody-producing plasma cells, differentiation of monocytes to macrophages, and growth of hematopoietic stem cells (for review, see Ref. 1). We have shown that IL-6 differentiates CD4+ T cells to effector T helper 2 cells (2, 3).

IL-6 binds to the cell surface IL-6R (IL-6Ra), causing its dimerization with signal-transducing element gp130/IL-6R (4–6). IL-6 can also bind to a soluble IL-6R (sIL-6Ra) to induce IL-6 signal transduction pathways (7). The gp130 element of the IL-6R is shared by the ciliary neutrophic factor, oncostatin M, leukemia inhibitory factor, IL-11, and cardiotoxin-1 receptors. Dimerization of the gp130 element in response to IL-6 leads to the activation of the MAPK signaling pathways, specifically, the extracellular signal-regulated kinases 1 and 2 (8). IL-6 signaling also leads to the activation of the transcription factor C/EBPβ [also defined as NFIL-6/IL-6DBP (9, 10)], which appears to be phosphorylated by MAPK (11). The expression of C/EBPβ (NFIL-6), another member of the C/EBP family of transcription factors, is induced in response to IL-6 (12). C/EBPβ can act synergistically with C/EBPα to increase the transcriptional activity of type 1 IL-6-responsive elements (13).

gp130 dimerization induces the phosphorylation of associated JAK family members JAK1 and JAK2 and Tyk2 (14–16). IL-6 has been shown to induce the tyrosine phosphorylation and nuclear translocation of STAT3 and, to a much lesser extent, STAT1. These transcription factors in turn bind to DNA and regulate the transcriptional activity of type 2 IL-6-responsive elements (17–19). It has been recently shown that the induction of C/EBPβ by IL-6 is regulated by STAT3 (20), suggesting that cross-talk exists between these two IL-6-mediated signaling pathways.

IL-6 has been involved in the growth of a variety of cancer cells (for review, see Ref. 1). It can act as a growth factor for myeloma/plasmacytoma, renal cell carcinoma, cervical carcinoma, AIDS Kaposi’s sarcoma-derived cells, and certain T- and B-cell lymphomas (21–26). Analysis of transgenic mice overexpressing IL-6 in B cells has shown that IL-6 is involved in the development of plasmacytoma and myeloma (1). Moreover, elevated IL-6 serum levels have been detected in patients with renal cancer, ovarian cancer, plasmacytoma, and myeloma (27).

In the present study, we demonstrate that IL-6 can be produced by breast cancer cells in an autocrine fashion that confers their resistance to drug treatment but does not affect their growth. The IL-6-induced drug resistance is associated with increased expression of the multidrug resistance gene, mdrl, and up-regulation of C/EBPβ and C/EBPδ. Because analysis of IL-6 expression shows that IL-6 can be produced by some tumor cells in breast cancer patients, it is possible that these IL-6-producing cancer cells are more resistant to chemotherapeutic agents.

MATERIALS AND METHODS

Cell Lines. MCF7 and MCF7/ADR cells were a gift of Dr. Ken Cowan (National Cancer Institute, Bethesda, MD). MCF7, MCF7/ADR, MCF7/IL-6A, and MCF7/IL-6B cells were maintained in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) containing 10% FCS (Life Technologies, Inc.). MCF7/ADR cells were maintained in the presence of 1 μM doxorubicin (Sigma Chemical Co., St. Louis, MO) to maintain the multidrug-resistant phenotype. Seven days before experiments were performed, MCF7/ADR cells were removed from doxorubicin-containing medium and maintained in doxorubicin-free medium as described previously (28, 29).

Recombinant human IL-6 (R&D Systems, Minneapolis, MN) was used to pretreat MCF7 cells. The cells were cultured in the presence of exogenous IL-6 (50 ng/ml) for 10 days. IL-6 was added to the culture every 2 days. After the pretreatment period, the cells were harvested, washed, and replated in the presence of IL-6, and their resistance to doxorubicin was determined by the MTT assay.

The human IL-6 cDNA plasmid (pBSF2.38.1) was kindly provided by Dr. Toshio Hirano (Biomedical Research Group, Osaka University Medical School, Osaka, Japan). The 1100-bp EcoRI-BamHI fragment obtained from pBSF2.38.1 was subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen, Carlsbad, CA). MCF7 cells were transfected using Lipofectin reagent (Life Technologies, Inc.) following the manufacturer’s protocol. Transfected cells were then plated in RPMI 1640 containing 10% FCS and 400

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3 The abbreviations used are: IL, interleukin; C/EBP, CCAAT enhancer-binding protein; MAPK, mitogen-activated protein kinase; JAK, Janus protein kinase; STAT, signal transducer and activator of transcription; MTT, microculture tetrazolium; P, p, p; glycoprotein; HPRT, hypoxanthine phosphoribosyltransferase; RT-PCR, reverse transcription-PCR; EMSA, electrophoretic mobility shift assay; IL-6R, IL-6 receptor.
μg/ml G418 (Life Technologies, Inc.) for 4 weeks, and stable transfectants were selected for G418 resistance. Selected clones were maintained in medium containing 400 μg/ml G418 and transferred to G418-free medium 1 week before the experiments.

MCF7, MCF7/ADR, MCF7/IL-6A, and MCF7/IL-6B cells were plated at equal densities (3.0 × 10^6 cells/well), and their proliferation rates were determined after 3 days by measurement of [³H]thymidine (Amersham Pharmacia Biotech Corp., Piscataway, NJ) incorporation.

Selection of Cells Expressing a Dominant Negative of C/EBP. MCF7/IL-6A cells were cotransfected with the pHOOK-2 plasmid (Invitrogen) and either the empty vector (pCMV) or the expression vector containing the dominant negative C/EBP mutant (pCMV-A-C/EBP) using Lipofectin reagent following the manufacturer’s protocol. The transfection media were replaced with RPMI 1640 containing 10% FCS, and the cells were left in culture for 48 h. The Capture-Tec pHOOK-2 system (Invitrogen) was then used to select for the transfected cells following the manufacturer’s protocol.

Cytokine Measurements. Cells were plated at equal densities (3.0 × 10^6 cells/well) in medium alone for 6 days. Supernatants were harvested at different time points, and IL-6 production was determined by ELISA. ELISA As were performed using a purified antihuman IL-6 MAb (2 μg/ml), the corresponding biotinylated antihuman IL-6 MAb (1 μg/ml; PharMingen, San Diego, CA), and horseradish peroxidase-conjugated avidin D (2.5 μg/ml; Vector Laboratories, Burlingame, CA) following the protocol recommended by the manufacturer (PharMingen). Recombinant human IL-6 (R&D Systems) was used as the standard.

RNA Isolation and Northern Blot Analysis. Total RNA was extracted using the Ultraspec RNA isolation system (Biotex Laboratories, Inc., Houston, TX). Total RNA (10 μg) was analyzed by Northern blot as described previously (30). Northern blots were hybridized with cDNA probes specific for human IL-6 and human HPRT labeled with [32P]dCTP (Amersham Pharmacia Biotech) using the Random Primer Kit (Stratagene, La Jolla, CA).

RT-PCR. Total RNA was extracted using the Ultraspec RNA isolation system (Biotex). The first-strand cDNA was obtained by reverse transcription as described previously (31). The reverse transcription mixture (1 μl) was assayed to detect human IL-6, HPRT, and mdr1 mRNA by RT-PCR. The primers used for the amplification of IL-6 have been described previously (32). The primers used for the amplification of HPRT were 5'-hHPRT (5'-GGACTTCCGATCTGATC-3') and 3'-hHPRT (5'-AGCCTTATAGC-3'). The primers used for the amplification of mdr1 have been described previously (33). For IL-6 mRNA quantification by real-time PCR, total RNA was extracted using the Ultraspec RNA isolation system (Biotex) and reverse-transcribed, and the cDNA was quantified using the TagMan system (Applied Biosystems, Foster City, CA). Values for IL-6 mRNA were normalized to the relative quantity of 18S RNA in each sample. The primer and probe sets used for the amplification of the IL-6 mRNA and the 18S RNA control were supplied by Applied Biosystems, kit numbers 4309894P and 4310893E, respectively.

MTT Assay. The dose response of the cell lines to doxorubicin was determined using the MTT assay as described previously (34). The cells were incubated in the presence of different concentrations of doxorubicin, vincristine, or Taxol (0–10 μM) for 4 days. On day 4, the remaining cells were stained with MTT (Sigma Chemical Co.), the cell supernatants were aspirated, and 100 μl of DMSO were added to each well. The plates were shaken for 5 min, and the absorbances were quantified at 540 nm using a spectrophotometer (Biotek, Colchester, VT). Absorbances were normalized, and LD₅₀ values were calculated by nonlinear regression. Normalized data and nonlinear regression curves were plotted graphically as a percentage of viable cells.

Nuclear Extracts and EMSAs. “Mini” nuclear extracts were made from 6 × 10⁶ cells as described previously (35, 36). Binding reactions for the EMSAs were performed using 2 μg of nuclear protein and a [α-³²P]dCTP-end-labeled double-strand oligonucleotide as described previously (31). The C/EBP oligonucleotide used in the reaction mixture was C/EBP sense (5'-GGACGTCACATTGCACAATCTTAATA-3'; Ref. 32). One μl/reaction of a nonspecific antiserum against glutathione S-transferase, a specific anti-C/EBPβ peptide rabbit antiserum (37), or an anti-C/EBPβ rabbit antiserum was used in supershift experiments. The anti-C/EBPβ polyclonal antiserum was raised against the NH₂-terminal portion (amino acids 1–188) of murine C/EBPβ.⁵

Cell Surface Staining. To determine the surface expression of gp130, MCF7 and MCF7/ADR cells were harvested, washed, and stained with a PE-conjugated antihuman gp130 MAb (PharMingen) or a PE-conjugated antihuman IgG1 MAb (PharMingen) as an isotype control and analyzed by flow cytometry.

RESULTS

Production of IL-6 by Multidrug-resistant Breast Cancer Cells. Elevated serum levels of IL-6 have been described in some breast cancer patients (38). In correlation with previous observations (39), we have found that some tumor cells within human primary breast cancer tissue produce IL-6.⁵ Here we examined the production of IL-6 by the breast cancer cell line MCF7 and the multidrug-resistant breast cancer cell line MCF7/ADR. MCF7 and MCF7/ADR cells were plated at equal densities, cell supernatants were harvested at different time points, and the production of IL-6 by these cancer cells was determined by ELISA. No IL-6 was detected in the supernatant from MCF7 cells (Fig. 1A). In contrast, high levels of IL-6 were detected in the supernatant from MCF7/ADR cells (Fig. 1A).

To determine whether the accumulation of IL-6 in the supernatant of the MCF7/ADR cells was due to the selective expression of IL-6 in these cells, we examined IL-6 gene expression by Northern blot analysis. High levels of IL-6 mRNA were present in MCF7/ADR cells, but no IL-6 mRNA was detected in MCF7 cells (Fig. 1B).

To confirm that the IL-6 gene is not expressed in MCF7 cells, we examined IL-6 gene expression in MCF7 and MCF7/ADR cells by RT-PCR. RT-PCR for HPRT was also performed as a positive control. In correlation with the results we obtained by Northern blot analysis, no IL-6 mRNA was detected in MCF7 cells despite expression of the HPRT gene (Fig. 1C). In contrast, high levels of IL-6 mRNA were detected MCF7/ADR cells, whereas the levels of HPRT mRNA were similar to those in MCF7 cells (Fig. 1C). Real-time PCR was also performed to quantify the levels of IL-6 gene expression in the MCF7/ADR cells. No IL-6 mRNA was detected in MCF7 cells, whereas high levels of IL-6 mRNA were present in MCF7/ADR cells (Fig. 1D). MCF7 cells fail to express the IL-6 gene, but expression of the IL-6 gene is significantly up-regulated in the drug-resistant MCF7/ADR cells.

Activation of C/EBPβ and C/EBPδ in Multidrug-resistant Breast Cancer Cells. The selective expression and secretion of IL-6 by MCF7/ADR cells revealed a potential association between the production of IL-6 and multidrug resistance. It was possible that the production of IL-6 could confer the resistance of MCF7/ADR cells to cytotoxic drugs by inducing IL-6 signal transduction pathways in an autocrine manner. We first examined the expression of the signal-transducing element of the IL-6R, gp130. MCF7 and MCF7/ADR cells were stained with a PE-conjugated anti-gp130 MAb and analyzed by flow cytometry. The histogram profiles depict the cell surface expression of the gp130 element on both MCF7 and MCF7/ADR cells (Fig. 2A). Comparable levels of gp130 were present on both MCF7 and MCF7/ADR cells (Fig. 2A), indicating that IL-6 was capable of mediating signals in both types of cells.

IL-6 activates the transcription factors C/EBPβ (NFIL-6) and C/EBPδ (NFIL-6β; Refs. 9, 10, 12, and 13). To determine whether the IL-6 pathway was activated in MCF7/ADR cells, the DNA binding of C/EBP transcription factors was determined by EMSA. Nuclear extracts from MCF7 and MCF7/ADR cells were incubated with an

⁴ C. A. Cantwell and P. F. Johnson, unpublished observations.
⁵ L. Weiss and M. Rincón, unpublished data.
oligonucleotide containing the consensus regulatory sequence recognized by the C/EBP family of transcription factors (32). Elevated levels of C/EBP DNA complexes were observed in MCF7/ADR cells as compared with MCF7 cells (Fig. 2B). To identify the components of the C/EBP complexes, we performed EMSA experiments in the presence of antiserum against the C/EBPβ and C/EBPδ transcription factors. Supershift analysis using anti-C/EBPδ antiserum showed high levels of C/EBPδ in the nucleus of MCF7/ADR cells, whereas no C/EBPδ was detected in MCF7 cells. Elevated levels of C/EBPβ were also present in the nucleus of MCF7/ADR cells as compared with MCF7 cells (Fig. 2B). These results demonstrate that this IL-6 signal transduction pathway is constitutively activated in MCF7/ADR cells characterized by the selective accumulation of C/EBPβ and C/EBPδ in the nucleus. Furthermore, these results suggest that MCF7/ADR cells undergo persistent IL-6-mediated signaling in an autocrine manner.

**IL-6 Confers Multidrug Resistance in MCF7 Cells.** Multidrug resistance is the broad spectrum resistance of human cancers to potent anticancer agents (40, 41). To date, the molecular mechanisms by which tumor cells become resistant to diverse groups of drugs remain unclear. The selective production of IL-6 by the MCF7/ADR cells suggested that IL-6 could play a role in the resistance of these cells to the cytotoxic activities of anticancer compounds. To address this hypothesis, MCF7 cells were cultured in the presence or absence of IL-6 for 10 days. After the pretreatment period, the cells were harvested, washed, and replated in the presence or absence of IL-6, and their resistance to doxorubicin was determined by the MTT assay (34). Pretreatment of MCF7 cells with IL-6 caused an 8–10 fold increase in resistance to doxorubicin (Fig. 3), indicating that the presence of exogenous IL-6 increased the resistance of breast cancer cells to doxorubicin treatment.

To determine whether the endogenous production of IL-6 by tumor cells could provide self-protection against drug-induced cell death, we constitutively expressed IL-6 in MCF7 cells and examined the effect of IL-6 expression on the drug resistance of these cells. MCF7 cells were transfected with an expression vector containing the human IL-6 gene. Stable MCF7 transfected (MCF7/IL-6) clones were isolated and screened for their ability to produce IL-6. Two representative clones that produced intermediate (MCF7/IL-6A) and high (MCF7/IL-6B) levels of IL-6 were used for subsequent studies.

**Fig. 1. IL-6 production by MCF7 and MCF7/ADR cell lines.** A, MCF7 and MCF7/ADR cells were plated in medium alone, and IL-6 production was determined by ELISA. B, total RNA was extracted from MCF7 and MCF7/ADR cells and examined for the expression of IL-6 and HPRT by Northern blot analysis. C, expression of IL-6 and HPRT was examined by RT-PCR using total RNA extracted from MCF7 and MCF7/ADR cells and primers specific for human IL-6 and HPRT. D, IL-6 mRNA levels in MCF7 and MCF7/ADR cells were determined using real-time PCR.
levels of IL-6 (Fig. 4A) were chosen for subsequent studies. The levels of IL-6 gene expression in the two stable transfected clones were also quantified by real-time PCR. In correlation with the secreted IL-6 levels, high levels of IL-6 mRNA were detected in the MCF7/IL-6B clone, and low levels of IL-6 mRNA were detected in the MCF7/IL-6A clone (Fig. 4B).

To determine whether the expression of IL-6 in MCF7 cells could increase their sensitivity to drug treatment, we examined the susceptibility or resistance of MCF7/IL-6A and MCF7/IL-6B cells to doxorubicin treatment using the MTT assay. Interestingly, MCF7/IL-6A and MCF7/IL-6B cells exhibited an approximately 70-fold increase in their resistance to doxorubicin as compared with MCF7 cells (Fig. 4C). Although the levels of IL-6 secreted by MCF7/IL-6A were lower than those secreted by MCF7/IL-6B, both cell lines were equally resistant to doxorubicin treatment, suggesting that low levels of IL-6 produced by tumor cells are sufficient to confer drug resistance. Stable MCF7 transfected clones that did not produce IL-6 exhibited similar drug sensitivity to MCF7 cells (data not shown).

To determine whether the endogenous production of IL-6 can confer resistance to other drugs associated with multidrug resistance, we examined the susceptibility or resistance of MCF7, MCF7/ADR, and MCF7/IL-6A cells to vincristine and Taxol (42). Similar to the results with doxorubicin, MCF7/ADR and MCF7/IL-6A cells exhibited increased resistance to both vincristine (Fig. 4D) and Taxol (Fig. 4E) as compared with MCF7 cells. In contrast, MCF7, MCF7/IL-6A,
whether the enhanced drug resistance of the MCF7/IL-6 cells was also expressed high levels of the membrane pump encoded for by the phenotype of cancer cells. P-gp is an energy-dependent transmembrane glycoprotein, P-gp, has been associated with the multidrug resistance against glutathione-site. The binding reactions were performed in the presence of nonspecific antiserum (A-C/EBP).

**Gene Expression by Activating C/EBP Transcription Factors.** The selective expression of a M, 170,000 transmembrane glycoprotein, P-gp, has been associated with the multidrug resistance phenotype of cancer cells. P-gp is an energy-dependent transmembrane pump encoded for by the mdr1 gene and is believed to reduce the intracellular accumulation of anticancer drugs (43–52). It has been previously demonstrated that MCF7/ADR cells, but not MCF7 cells, express high levels of mdr1 and its gene product, P-gp (45). To test whether the enhanced drug resistance of the MCF7/IL-6 cells was also associated with the presence of P-gp, we examined mdr1 expression in MCF7, MCF7/ADR, MCF7/IL-6A, and MCF7/IL-6B cells by RT-PCR. In correlation with previous studies (45), no mdr1 gene expression was detected in the MCF7 cells, but high levels were expressed in MCF7/ADR cells (Fig. 5A). Interestingly, high levels of mdr1 mRNA were found in MCF7/IL-6A and MCF7/IL-6B cells (Fig. 5A). These data indicate that the autocrine production of IL-6 induces mdr1 gene expression in breast cancer cells. P-gp protein levels were also elevated in MCF7/IL-6A and MCF7/IL-6B cells (data not shown).

Previous reports have shown that a C/EBP consensus binding site is present in the mdr1 promoter region and is able to mediate mdr1 gene transcription (53, 54). In correlation, our results showed increased levels of C/EBP complexes present in the MCF7/ADR (Fig. 2B) cells that express mdr1. To determine whether the increase in mdr1 expression in MCF7/IL-6A and MCF7/IL-6B cells was due to the activation of C/EBP transcription factors by IL-6, we examined the DNA binding of C/EBP transcription factors by EMSA. Similar to MCF7/ADR cells, high levels of C/EBP complexes were present in the nucleus of MCF7/IL-6A and MCF7/IL-6B cells as compared with MCF7 cells (Fig. 5B). Supershift analysis using anti-C/EBPβ and anti-C/EBPβ antisera showed high levels of nuclear C/EBPβ in MCF7/IL-6A and MCF7/IL-6B cells, whereas no C/EBPβ was detected in MCF7 cells. Moreover, the levels of nuclear C/EBPβ in MCF7/IL-6A and MCF7/IL-6B cells were substantially higher than those present in MCF7 cells (Fig. 5B). These results suggest that the autocrine production of IL-6 by breast cancer cells activates C/EBP transcription factors, induces the expression of the mdr1 gene, and increases cell resistance to drug treatment.

To determine whether a causal relationship exists between the high amounts of C/EBP in the nuclei of the IL-6-producing MCF7 clones and the induction of mdr1 gene expression, we examined the effect of C/EBP inhibition on mdr1 gene expression in the MCF7/IL-6A cells. We performed transient transfections with a mutant form of C/EBP (A-C/EBP) that acts as a dominant negative for endogenous C/EBP (55, 56). MCF7/IL-6A cells transfected with either the A-C/EBP or the empty vector were isolated with the pHOOK-2 system. RNA was extracted, and mdr1 gene expression was determined by RT-PCR. Compared with the levels of mdr1 in cells transfected with the empty vector, expression of the A-C/EBP mutant in the MCF7/IL-6A cells caused a reduction of mdr1 gene expression (Fig. 5C). The partial inhibition of mdr1 gene expression by A-C/EBP correlates with the partial inhibitory effect of this mutant on the endogenous C/EBP DNA binding (data not shown). Thus, IL-6 up-regulates mdr1 gene by activation of the C/EBP signaling pathway.

**DISCUSSION**

IL-6 is a pleiotropic cytokine that plays a significant role in the growth and differentiation of cells. Several studies have addressed the role of IL-6 in tumor cell growth, but its exact role remains varied and unclear. It appears that the effect of IL-6 on tumor cell growth may depend on the tumor cell type. In this study, we demonstrate for the first time that IL-6 plays a new role in cancer biology; it promotes multidrug resistance.

Chemotherapy has been one of the most effective and widely used means of treating cancer. The development of multidrug resistance, however, has posed major obstacles to the efficacy of chemotherapy and hence cancer treatment (57). Here, we show that IL-6 is expressed and secreted by multidrug-resistant breast cancer cells, whereas no IL-6 gene expression is found in the parental drug-sensitive cells. Despite the expression of a large number of genes selectively expressed in multidrug-resistant cells, only a few of these genes have been shown to increase resistance to chemotherapeutic drugs (for review, see Ref. 58). We demonstrate that the expression of IL-6 in non-IL-6-producing breast tumor cells induces drug resistance, indicating that the production of IL-6 protects the cells from cytotoxic agents. Other studies have shown that inhibition of IL-6 secretion in prostate cancer cell lines increases the sensitivity of these cells to
anticancer drugs (59). Thus, some tumor cells may acquire the ability to express and produce IL-6 as a protective mechanism against drug-induced death. The stimuli responsible for the constitutive expression of IL-6 in multidrug-resistant cells remain to be determined.

In addition to the effect of endogenous production of IL-6, we have also shown that a relatively short period of treatment with exogenous IL-6 increases resistance of the MCF7 drug-sensitive cells to drug treatment but does not affect cell growth. These results suggest that administration of IL-6 to breast cancer patients could increase the survival of tumor cells during chemotherapy. Despite this prediction, IL-6 has been administered (Phase I/II clinical trials) to breast, ovarian, myeloid leukemic, and metastatic cancer patients in combination with chemotherapy. The principal rationale for administering IL-6 during these studies was to stimulate platelet growth and differentiation (60, 61), although no significant effects on platelet numbers were observed (61, 62). Other clinical studies have also used IL-6 as an antitumor agent in the treatment of renal cell carcinoma and melanoma patients (63–65). It is possible that treatment with IL-6 contributed to the development of multidrug resistance in those patients.

The first and most widely studied factor known to modulate multidrug resistance is P-gp. P-gp is encoded by the mdr1 gene and is believed to mediate multidrug resistance by reducing the intracellular accumulation of cytotoxic drugs and compounds. Many types of cancers express high levels of mdr1 and are known to be unresponsive to chemotherapy (66–68). In fact, P-gp expression has been considered to be a predictive factor of poor response to chemotherapy and overall survival (69–71). Here we demonstrate that expression of IL-6 in drug-sensitive breast cancer cells induces mdr1 gene expression and, in correlation, increases the resistance of the cells to doxorubicin treatment. Thus, the induction of mdr1 gene expression is a potential mechanism by which IL-6 provides drug protection.

Specific regulatory elements have been identified within the 4.7-kb promoter region of the mdr1 gene (for review, see Ref. 72; see also Refs. 73 and 74), but the relative contribution of these elements to mdr1 gene expression remains unclear. There is a C/EBPβ/NFIL-6–specific regulatory element located between −157 and −126 bp in the mdr1 promoter (53). It has been shown that in response to phorbol 12-myristate 13-acetate, C/EBPβ, a downstream component of the IL-6 signal transduction pathway (10), can bind and transactivate the human mdr1 gene promoter (53, 54). C/EBPβ, another member of the C/EBP family of transcription factors, has been shown to act synergistically with C/EBPβ from C/EBP consensus sequences and is also regulated by the IL-6 signal transduction pathway (13). Here we demonstrate that the induction of mdr1 gene expression by IL-6 in breast cancer cells correlates with the activation of C/EBPβ and C/EBPδ. Thus, the constitutive expression of the mdr1 gene in multidrug-resistant cells can be maintained by activated C/EBPβ and C/EBPδ. We propose that some tumor cells may acquire the ability to express and produce IL-6. This ability may in turn act as a protective mechanism against chemotherapeutic drugs by activating C/EBPβ/C/EBPδ and the expression of the mdr1 gene. STAT3 is an additional transcription factor in the IL-6 signal transduction pathway (for review, see Ref. 1). After the treatment of cells with IL-6, gp130 dimersize, and the gp130-associated JAK1 and JAK2 phosphorylate STAT3 (14, 15). Recently, it has been demonstrated that constitutively activated STAT3 leads to the transformation of cells, suggesting that STAT3 is an oncogenic factor (75). In our study, we show that the expression of IL-6 in non-IL-6-producing breast cancer cells does not affect cell growth but does increase drug resistance. It is therefore possible that activation of the JAK/STAT signaling pathway in cells treated with IL-6 leads to the transformation of cells, whereas activation of the MAPK/C/EBP signaling pathway by IL-6 increases resistance to the cytotoxic activities of specific agents. Interestingly, the induction of the MAPK signaling pathway by IL-6 requires ErbB2/neu, a growth factor receptor implicated in the neoplastic transformation of prostate carcinoma cells, whereas activation of the JAK/STAT pathway is independent of ErbB2 (76). Increased expression of ErbB2 has also been correlated with the resistance of cells to cytotoxic drugs (77–80).

In correlation with other studies (39), we have observed that some tumor cells within primary breast cancer tissue have acquired the ability to produce IL-6. Additional studies need to be performed to ascertain whether a correlation exists between the presence of IL-6-producing breast cancer cells and the response of patients to chemotherapy. IL-6 could therefore be used as a predictive factor for the development of multidrug resistance and poor response to chemotherapy. Consistent with this hypothesis, elevated IL-6 serum levels have been found in ovarian cancer patients who do not respond to chemotherapy (81). Elevated levels of IL-6 in serum have also been correlated with prostate metastasis and morbidity (82–84). It is clear that IL-6 should not be provided to cancer patients as a therapeutic agent, and drugs used to inhibit the expression of IL-6 or the IL-6 signal transduction pathway may be beneficial.

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