The role of oncogenes in drug resistance

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Abstract

Increasing evidences indicate that oncogenes can directly or indirectly impact on cancer-cell drug resistance. This chapter provides a conceptual review regarding the role of oncogenes in drug resistance. The review is focused on drug resistance mediated by oncogenes encoding growth factor receptors, signaling molecules, transcription factors, cell-cycle regulators, and apoptosis regulators. It is my hope that better understanding on the role of oncogenes in drug resistance will invoke ideas on new approaches to enhance the cytotoxicity of the standard chemotherapeutic agents by functional perturbation of resistance-inducing oncogenes.

Introduction

Tumor-cell resistance to chemotherapeutic agents is a well-recognized phenomenon and is one of the most serious problems in cancer management. Our understanding of the molecular basis of tumor-cell drug resistance has advanced enormously in the past few decades. The observation that malignant cells commonly become resistant to therapy while normal cells rarely do suggests that certain molecular processes involved in malignancies, (e.g., oncogene activation) may play a role in drug resistance.

Although oncogenes are defined as genes with oncogenic potential, they do play important functions in normal cells. In the past 20-plus years, more than 70 oncogenes that positively activate cell growth have been identified (Marx, 1994). Among these oncogenes, some encode membrane growth factor receptors that pick up growth factor signals, some are cytoplasmic signaling molecules that transmit growth signals, and some are nuclear transcription factors that function as responders to growth signals. Recent studies on cell-cycle control revealed that deregulated cell-cycle regulators can function as oncogenes. In addition, certain oncogenes may not have significant impact on cell growth but can block programmed cell death (apoptosis), thereby contributing to tumor pro-

gression. In this chapter, the new findings regarding the types of oncogenes involved in drug resistance will be reviewed. The rapid development seen in this subject and its potentially tremendous influence on cancer management deserve a detailed discussion. My intention, however, is not to review this issue exhaustively, but to present some examples of the roles of oncogenes in drug resistance. From these examples, one can develop a general concept regarding the role of oncogenes in drug resistance.

Growth Factor Receptors and Drug Resistance

It has recently been recognized that activation of growth factor receptors can not only perturb growth regulation but may also affect the sensitivity of cancer cells to chemotherapy.

A prototype of growth factor receptors is *c-erbB2*. The *c-erbB2* gene encodes a 185-kDa transmembrane glycoprotein (p185) that belongs to the epidermal growth factor receptor (EGFR, or *erbB1*) family (Bargmann *et al.*, 1986; Yamamoto *et al.*, 1986; Yarden and Weinberg, 1989). The *c-erbB2*-encoded tyrosine kinase receptor shares extensive sequence homology with EGFR, and the amino acids in the tyrosine kinase domain of the two receptors are more

than 80 percent identical (Bargmann *et al.*, 1986; Coussens *et al.*, 1985; Schechter *et al.*, 1985; Yamamoto *et al.*, 1986). Although the ligand for the *c-erbB2* receptor has not been identified, the *c-erbB2* receptor is known to mediate lateral signal transduction of all *erbB*-receptor family members (Carraway *et al.*, 1994; Graus-Porta *et al.*, 1997; Plowman *et al.*, 1993), because the *c-erbB2* receptor is the preferred heterodimerization partner for all *erbB* receptors. After ligand binding, EGFR, *erbB3*, and *erbB4* can heterodimerize with *erbB2* and can lead to the tyrosine phosphorylation of all of these receptors (Graus-Porta *et al.*, 1997; Sliwkowski *et al.*, 1994; Wallasch *et al.*, 1995).

The *c-erbB2* gene has been found to be amplified, overexpressed, or both in approximately 30 percent of all human breast carcinomas (Slamon *et al.*, 1987). Individuals with such carcinomas were shown to have a significantly lower overall survival rate and a shorter time to relapse than did patients whose tumors did not overexpress *c-erbB2*. Interestingly, a clinical study has demonstrated that breast tumors that overexpress *c-erbB2* are less responsive to adjuvant chemotherapy regimens containing cyclophosphamide, methotrexate, and 5-Fluorouracil (CMF) than tumors that express a normal amount of *c-erbB2* (Gusterson *et al.*, 1992). Patients with node-positive early breast tumors that overexpressed *c-erbB2* were reported to benefit from higher doses of chemotherapy, whereas patients with early-stage disease that lacked *c-erbB2* expression did not receive this benefit from intensive therapy (Muss *et al.*, 1994), further suggesting a possible relationship between chemosensitivity and p185 expression in breast cancers. In addition, *c-erbB2* oncoprotein expression was found to be useful in predicting survival time only for patients receiving adjuvant therapy, further suggesting that it may be a marker of drug resistance (Tetu and Brisson, 1994). On the other hand, there was a controversial report of 81 cases of primary breast carcinoma treated with adjuvant chemotherapy with a median follow-up of 5 years; no significant trend was observed in response to adjuvant chemotherapy in correlation with *c-erbB2* expression, *p53* expression, or both when the expression of the *c-erbB2* and *p53* proteins was examined by immunohistochemistry (Jacquemier, 1994).

In addition to these clinical investigations of *c-erbB2* expression and chemosensitivity, experimental data from laboratory studies have provided more direct evidence that *c-erbB2* overexpression can confer drug resistance of cancer cells. Tsai and coworkers re-

ported a statistically significant correlation ($r = 0.67$ to 0.86 ; $P < 0.005$) between the IC_{50} values of doxorubicin, carmustine, cisplatin, melphalan, mitomycin, and etoposide and the degree of *c-erbB2* gene expression in a panel of 20 non-small cell lung carcinoma (NSCLC) cell lines obtained from untreated patients (Tsai *et al.*, 1993). Furthermore, NSCLC cell line H460 transfected with *c-erbB2* cDNA demonstrated enhanced expression of p185, which led to intrinsic chemoresistance to doxorubicin, cisplatin, mitomycin, and etoposide (Tsai *et al.*, 1995). In addition, several studies have demonstrated that anti-p185^{*c-erbB2*} antibodies are synergistic with chemotherapeutic drugs (such as cisplatin) in inhibiting breast and ovarian cancer-cell growth *in vitro* (Arteaga *et al.*, 1994; Hancock *et al.*, 1991; Pietras *et al.*, 1994).

To examine the relationship between overexpression of the *c-erbB2*-encoded p185 protein and increased drug resistance in human breast cancer cells, we tested a panel of established human breast cancer cell lines that express p185 at different levels for their sensitivity to the chemotherapeutic agent paclitaxel (Taxol), which is a potent antineoplastic agent isolated from the stem bark of the Pacific yew tree (Wall and Wani, 1995; Wani *et al.*, 1971) and has been shown to be highly effective in treating advanced metastatic breast cancers in several phase-I and phase-II clinical trials (Chevalier *et al.*, 1995; Holmes *et al.*, 1991). We found that higher levels of p185 expression in these cell lines correlated with increased resistance to Taxol and docetaxel (Taxotere). N29 monoclonal antibodies that can specifically downregulate p185 sensitized the p185-overexpressing breast cancer cells to Taxol (Yu *et al.*, 1997). To further investigate whether p185 overexpression can lead to increased resistance to Taxol, we transfected the human *c-erbB2* cDNA into the very low p185-expressing MDA-MB-435 human breast cancer cells and examined sensitivity to Taxol among the parental MDA-MB-435 cells and stable transfectants (named 435.eB) with increased expression of p185. The p185-overexpressing 435.eB transfectants were more resistant to Taxol than the parental cells. The increased resistance to Taxol and Taxotere was found only in *c-erbB2*-transformed cells and not in *ras*-transformed cells when oncogene-transformed NIH3T3 cells were examined. Our data demonstrated that overexpression of p185 can lead to intrinsic Taxol resistance independent from oncogenic transformation as well as from *mdr-1* mechanisms (Yu *et al.*, 1996).

Several studies indicated that another tyrosine kinase receptor, EGFR, may also play a critical role

in cancer-cell chemosensitivity. Overexpression of EGFR correlates with a poor prognosis in many different types of cancers. Several recent studies have placed the EGFR prominently in the signal transduction pathway, demonstrating that EGFR signaling may have an important role throughout the course of malignant progression (Chrysogelos and Dickson, 1994). The role of EGFR in chemosensitivity was implicated by the observation that human breast cancer cell lines selected for multidrug resistance (MDR) displayed increased EGFR (Dickstein *et al.*, 1993; Vickers *et al.*, 1988). Anti-EGFR monoclonal antibody 108, an EGFR-blocking agent, enhanced the antitumor activity of *cis*-diamminedichloroplatinum (*cis*-DDP) at the time of carcinoma-cell implantation (Aboud-Pirak *et al.*, 1988), which further supported the role of EGFR in chemoresistance. In addition, combination therapy consisting of anti-EGFR monoclonal antibody 225 (or 528) plus *cis*-DDP resulted in substantial growth inhibition of the A431-cell xenograft compared with that of an untreated control group and animals receiving either monoclonal antibodies alone or *cis*-DDP alone (Fan *et al.*, 1993). These studies suggest that overexpression of EGFR may lead to chemoresistance in these cancer cell lines and that receptor-blocking agents may represent a new treatment approach that can sensitize EGFR-overexpressing cancer cells to chemotherapeutic agents. However, there are also conflicting reports that reduced expression of EGFR caused by the introduction of EGFR antisense oligonucleotides was associated with increased resistance to *cis*-DDP in MDA-MB-468 human breast cancer cells, which express high levels of EGFR (Dixit *et al.*, 1997).

One possible explanation for the dilemma about the role of EGFR or *c-erbB2* in drug resistance is that the effects of EGFR or *c-erbB2* in chemosensitivity may be specific to particular tumor cell lines, cell types, or drugs (Mendelsohn and Fan, 1997). The dilemma also suggests that the need exists for future large-scale clinical studies with sufficient statistical power to reliably confirm the value of EGFR and *c-erbB2* as markers of resistance to different chemotherapeutic agents and regimens in different types of cancers.

Signaling Molecules and Drug Resistance

The interactions between various molecular steps in signal transduction pathways and chemotherapeutic

agents are emerging from recent intensive studies. Various oncogenes in signaling pathways have been indicated to contribute to MDR, including *ras*, protein kinase C (PKC), *v-mos*, *src*, etc. (Aggarwal *et al.*, 1994; Kopnin *et al.*, 1995; Masumoto and Nakano, 1997).

The *ras* oncogene is an archetype of the cytoplasmic signaling connectors. One of the first oncogenes to be directly linked to human cancers, *ras* is one of the most commonly activated oncogenes that contributes to the tumorigenesis of approximately 30% of all human cancer (Marx, 1994).

Aberrant expression and mutations of various *ras* oncogenes have been implicated in the poor prognosis of and drug resistance in human cancers. It was reported that PC3(R) human prostate cancer cell line, which is a multidrug-resistant variant of the PC3 cell line, demonstrated increased resistance to VP-16, m-amsacrine (m-AMSA), doxorubicin, vincristine (VCR), choline phosphotransferase (CPT), *cis*-diamminedi-chloroplatinum (CDDP), and melphalan compared with the PC3 cells (Yamazaki *et al.*, 1994). The drug resistant PC3(R) cells expressed H-*ras* at a significantly higher level than the chemosensitive PC3 cells, whereas the expression or activity of *mdr-1*, topoisomerase I, topoisomerase II, and glutathione-S-transferase (GST) were not different in these cell lines (Yamazaki *et al.*, 1994). The results indicate that the *ras* gene may play a role in the emergence of *de novo* drug resistance in PC3(R) cells.

Riva and coworkers investigated the effect of varying levels of H-*ras* expression and the presence of mutation on resistance to 1-beta-D-arabinofuranosylcytosine (ara-C) in rodent Rat-1a fibroblasts and human mammary HBL100 cells (Riva *et al.*, 1995). They found that transfection of cells by H-*ras* renders cells resistant to ara-C because of the inactivity of deoxycytidine kinase and the decrease of the gene's mRNA expression. The degree of ara-C resistance correlated directly with the level of H-*ras* expression (Riva *et al.*, 1995). These findings suggest that H-*ras* levels may regulate the metabolic pathways of drugs such as ara-C and hence influence the therapeutic success of ara-C in some tumors.

Interestingly, when three bladder carcinoma cell lines (one parental, one transfected by wild-type H-*ras*, and one transfected by mutant H-*ras*) were cultured as three-dimensional tissue on collagen-sponge gels, the *ras* expression level or mutational status did not affect drug resistance (Theodorescu *et al.*, 1993). In another study, ten bladder tumors exposed to *cis*-

platin and eight untreated bladder tumors were examined for *ras* point mutations using polymerase chain reaction (PCR) and allele-specific oligohybridization, it was found that only one of the ten treated tumors and none of the untreated tumors harbored a *ras* point mutation (Wood *et al.*, 1992). Therefore, *ras* point mutations occur infrequently in bladder tumors and do not appear to correlate with cisplatin resistance *in vivo* (Wood *et al.*, 1992). The effect of the *ras* oncogene on bladder-cancer drug resistance seems to be minimal.

Nevertheless, the *ras* oncogene is believed to have an effect on drug resistance in other cell types and has been shown to activate reporter-gene expression driven by human and rodent *mdr-1* gene promoters (Kopnin *et al.*, 1995). The *ras* can also upregulate expression of endogenous *mdr-1* mRNA in rodent immortalized fibroblasts and enhance P-glycoprotein activity, which contributes to the resistance of cells to the cytotoxic action of colchicine and other drugs (Kopnin *et al.*, 1995). These findings provide additional support to the role of *ras* in drug resistance and revealed a molecular mechanism of *ras*-induced drug resistance.

Transcription Factors and Drug Resistance

The ultimate target of many growth signal pathways is the transcriptional activation of a specific set of genes by transcription factors, which allow cells to react to environmental changes. Because chemotherapeutic drugs can impact cell growth and interact with various components of growth-signal pathways, it is not surprising that transcription factors may regulate cellular response to chemotherapeutic drugs.

A typical class of transcription factors that serves as the nuclear target of many growth-signal pathways is the activator protein-1 (AP-1) family (Ransone and Verma, 1990), which includes members of the *fos* and *jun* families. Members of the *fos* family form heterodimers with *jun* proteins and regulate transcription by binding at the AP-1 DNA binding sites, which are found in the regulatory sequences of many AP-1-dependent genes involved in cell proliferation, differentiation, tumorigenesis, and possibly drug resistance.

Several studies indicated that AP-1 (*jun*, *fos*) can induce GST expression that leads to cancer chemoprotection and drug resistance (Hayes and Pulford, 1995). Disruption of the putative AP-1 response element within GSTP1 (an isoenzyme that belongs to the GST family) promoter abrogated GSTP1 transcription

in MCF-7 breast cancer cells (Moffat *et al.*, 1994). An AP-1 sequence also exists in the promoter region of the *mdr-1* gene, and expression of *c-fos* can be induced by cytotoxic drugs in multidrug-resistant human oral epidermoid carcinoma (KB) cells and mouse sarcoma 180 cells that already express high levels of *c-jun* (Bhushan *et al.*, 1992). These data suggest that *c-fos* may act in concert with *c-jun* to control transcription of *mdr-1* by binding to the specific AP-1 regulatory site (Bhushan *et al.*, 1992), thereby conferring resistance to vinblastine, doxorubicin hydrochloride (Adriamycin), colchicine, and VP-16 in these cells. AP-1 transcription factors have also been reported to contribute to the MDR of human prostate cancer cells (Yamazaki *et al.*, 1994), and resistance to 5-fluorouracil and cisplatin in stage-II to stage-IV squamous cell carcinoma of the head and neck (HNSCC) (Riva-Lavieille, 1994). Interestingly, when a hammerhead ribozyme designed to cleave *fos* RNA was transfected into multi-drug-resistant A2780AD human ovarian carcinoma cells, it resulted in decreased expression of *c-fos*, which in turn to decreased expression of *mdr-1* gene (Scanlon *et al.*, 1994). The ribozyme transformants displayed altered morphology and restored sensitivity to chemotherapeutic agents (Scanlon *et al.*, 1994). These results demonstrated that *c-fos* plays a central role in drug resistance.

Another class of transcription factors that may be involved in drug resistance is the myc protein family (L-myc, N-myc, c-myc). The myc protein forms a sequence-specific DNA-binding complex with max and acts as a transcription factor; thus, it may be possible that *myc* oncogenes are involved in DNA synthesis and repair processes, mediating drug resistance. For example, L-myc was suggested to be involved in the transition from drug sensitive to drug resistant phenotype of small cell lung cancer cell lines (Tanio, 1992).

N-myc amplification correlates with a poor prognosis in neuroblastoma patient and amplified N-myc confers drug resistance to certain agents used for neuroblastoma treatment. Studies involving the treatment of multiple human neuroblastoma cell lines with a range of N-myc gene copy numbers using cisplatin/VP-16 have shown a significant correlation between resistance to cisplatin and VP-16 and N-myc copy numbers (Livingstone and Mairs, 1997). These results indicate that N-myc amplification may be responsible for treatment failure in patients receiving cisplatin or VP-16. In another study, N-myc amplification was found to correlate with expression of the gene

for the multidrug-resistance-associated protein (MRP) in neuroblastoma cells (Norris *et al.*, 1996). Because overexpression of MRP has been linked with MDR *in vitro*, the results suggest that N-*myc* may mediate drug resistance by the MRP pathway.

Amplification and overexpression of *c-myc* in small cell lung carcinoma (SCLC) is correlated with a poor prognosis. It was found that downregulation of endogenously expressed *c-myc* in the human SCLC cell line GLC4 and its CDDP- and Adriamycin-resistant sublines (GLC4-CDDP and GLC4-Adr) with an antisense oligodeoxynucleotide of the *c-myc* mRNA reduced *c-myc* protein expression and induced growth inhibition of 30 to 35% in all three cell lines (Van Waardenburg *et al.*, 1996). It resulted in increased CDDP sensitivity in GLC4-CDDP but not in GLC4. However, this *c-myc* antisense oligodeoxynucleotide did not affect Adriamycin sensitivity in all three lines (Van Waardenburg *et al.*, 1996). These findings suggest that *c-myc* plays a role in resistance to CDDP. Consistent with this notion, combination treatment with the *c-myc* antisense oligonucleotide plus CDDP was shown to overcome the resistance to CDDP of urinary bladder tumor cells; also, the increased intracellular accumulation of CDDP by the *c-myc* antisense oligonucleotide may play a role in the enhanced cytotoxicity obtained (Mizutani *et al.*, 1994). The effect of varying the level of *c-myc* expression on resistance to cisplatin was also examined in murine erythroleukemia cells expressing varying levels of *c-myc* gene product. It was found that the degree of cisplatin resistance correlated directly with the level of *c-myc* expression, and induction of *c-myc* in an inducible expression system significantly increased resistance to cisplatin (Sklar and Prochownik, 1991). Taken together, these findings suggest that *c-myc* levels may influence therapeutic success in some tumors and may regulate specific processes by which cells cope with DNA damage caused by DNA cross-linking agents.

Cell – Cycle Regulators and Drug Resistance

Many chemotherapeutic agents elicit cytotoxic function by targeting at specific cell-cycle stages during tumor-cell proliferation. It is conceivable that alterations in the expression of genes that control the cell cycle may be of critical importance in determining the chemosensitivity of tumor cells.

The key regulators of the cell-division cycle are cyclins and their catalytic partners, cyclin-dependent kinases (cdks). The effects of alterations in cyclins or cdks on drug action are beginning to be appreciated. For example, the effect of cyclin D1 overexpression on drug sensitivity in a human fibrosarcoma cell line has been systematically investigated (Hochhauser *et al.*, 1996). Cyclin D1 is expressed early in the G₁ phase, it associates with cdk4 and cdk6, and it mediates the phosphorylation and functional inactivation of the retinoblastoma protein (pRb), thereby promoting a transition from G₁ phase to S phase. Elevated levels of expression of the cyclin D1 protein have been found in a variety of cancers, including breast cancer, head and neck cancer, non-small-cell lung cancer, and mantle cell lymphomas (Bartkova *et al.*, 1995; Gillett *et al.*, 1994). Therefore, cyclin D1 is regarded as an oncogene for these types of tumors (Bates and Peters, 1995). Hochhauser and coworkers transfected a human cyclin D1 expression vector into the human HT1080 fibrosarcoma cell line and identified two transfectants that expressed high levels of cyclin D1 mRNA and protein as compared with control cells. As expected, a relative increase in the phosphorylated form of pRb and an increased fraction of cells in the S and G₂ phases of the cell cycle were observed in cells expressing high versus low levels of cyclin D1 (Hochhauser *et al.*, 1996). Interestingly, the cyclin-D1-overexpressing clones had increased dihydrofolate reductase (DHFR) mRNA and protein expression, which paralleled a statistically significant ($P < 0.01$) increase in resistance to methotrexate in cells expressing high levels of cyclin D1 compared with cells expressing lower levels (Hochhauser *et al.*, 1996). However, there was no difference in the resistance to doxorubicin, Taxol, and cytarabine among cells that express different levels of cyclin D1 (Hochhauser *et al.*, 1996). The data indicated that overexpression of cyclin D1 in HT1080 fibrosarcoma cells led to an increase in DHFR expression that resulted in increased resistance to methotrexate but had no effect on sensitivity to other classes of anticancer agents. Therefore, tumors with overexpression of cyclin D1 may be relatively refractory to methotrexate treatment.

These studies indicate that alterations in cell-cycle genes may differ in their effects on cytotoxicity. A recent study examined the effects of increased activity and expression of cyclin A, and cdk2, and cdk4 proteins on increased resistance to staurosporine, a potent protein kinase inhibitor (Chang *et al.*, 1996). Although no clear correlation was found in this study,

the effects of alterations of these and other cell-cycle regulatory genes on the responses of tumor cells to specific classes of drugs definitely deserve further investigation.

Apoptosis Regulators and Drug Resistance

Apoptosis is a new concept that could be of great importance in the understanding and treatment of cancer. It has been suggested that genes that regulate apoptotic cell death may play an important role in determining the sensitivity of tumor cells to chemotherapy. Resistance to apoptosis plays an important role in malignancies that are refractory to chemotherapy.

Inhibitors of apoptosis can induce resistance to chemotherapeutic drugs. The most well-known apoptosis inhibitor is *bcl-2*. The *bcl-2* protein p26^{*bcl-2*} is an inner-mitochondrial-membrane protein (Hockenbery *et al.*, 1990) that functions in an antioxidant pathway (Hockenbery *et al.*, 1993). This property could explain the antiapoptotic activity of *bcl-2* because peroxides seem to be important mediators for apoptosis (Hockenbery *et al.*, 1993). Despite the fact that (a) *bcl-2* expression was not an indicator of *in vitro* response to drugs in chronic lymphocytic leukemia (CLL) (Begleiter *et al.*, 1996; Morabito *et al.*, 1997); (b) *bcl-2* expression was not associated with resistance to EPOCH (infusional etoposide, vincristine, and doxorubicin and a bolus of prednisone and cyclophosphamide) chemotherapy in non-Hodgkin's lymphoma (NHL) (Wilson *et al.*, 1997) and (c) no differences in *bcl-2*, *bcl-x* and *bax* gene expression were observed in a panel of lung cancer cell lines derived from untreated and treated patients that exhibited different levels of chemosensitivity (Reeve *et al.*, 1996), numerous studies have demonstrated that the *bcl-2* protein is capable of preventing apoptosis induced by different drugs in different cell types and hence may be involved in drug resistance to multiple chemotherapeutic agents in a variety of cancer-cell types (Reed, 1995). Generally, when *bcl-2* is overexpressed, cells are less sensitive to cytotoxic drugs; on the contrary, when *bcl-2* is under-expressed, cells are more sensitive to cytotoxic drugs. In addition, clinical investigations revealed that *bcl-2* expression is associated with a poor prognosis and correlated with a poor response to therapy in several cancer types (Hill *et al.*, 1996).

Overexpression of the antiapoptosis *bcl-2* or *bcl-XL* protein relative to the proapoptosis *bax* protein has been recognized as the distal mechanism of MDR in

human acute myeloid leukemia (AML) cells. Enforced expression of the *bcl-2* or *bcl-XL* proteins in human AML HL-60 cells by transfection and overexpression of the *bcl-2* (HL-60/*bcl-2*) or *bcl-XL* (HL-60/*bcl-XL*) genes significantly reduced paclitaxel-induced apoptosis and led to the loss of cell viability without affecting intracellular accumulation of paclitaxel (Huang *et al.*, 1997). Resistance to paclitaxel-induced apoptosis and cytotoxicity was also reported in pre-B human leukemia 697 cells and their counterparts, which have been transfected with and overexpress the *bcl-2* gene (Tang *et al.*, 1994).

The relevance of *bcl-2* to drug resistance has been further demonstrated by antisense (As) approaches to achieve reduced *bcl-2* expression in t(14;18)-containing human NHL cell lines (Kitada *et al.*, 1994). Both synthetic *bcl-2*-As oligonucleotides and inducible expression plasmids that produce *bcl-2*-As transcripts induced reductions in *bcl-2* expression, resulting in a marked enhancement in the sensitivity of the lymphoma cells to conventional chemotherapeutic drugs such as ara-C and methotrexate (Kitada *et al.*, 1994). These results suggest that *bcl-2* antisense oligonucleotides are able to reverse apoptosis inhibition and sensitize cancer cells to chemotherapy.

The MCF-7 human breast cancer cells transfected with *bcl-2* expression plasmids displayed a marked increase in resistance to Adriamycin (Teixeira *et al.*, 1995). In this estrogen-receptor-positive cell line, *bcl-2* expression can also be upregulated by estrogen which confers resistance to Adriamycin (Teixeira *et al.*, 1995). Depletion of estrogen from the medium or addition of antiestrogen ICI 164,384 to the medium results in loss of expression of *bcl-2* mRNA and markedly lower resistance to Adriamycin compared with cells treated with estrogen alone (Teixeira *et al.*, 1995). Estrogen may thus promote resistance to chemotherapeutic drugs in estrogen-receptor-positive human breast cancer cells through a mechanism that involves regulation of the *bcl-2* proto-oncogene, which may offer an additional survival advantage for estrogen-dependent breast cancer cells in the presence of estrogen *in vivo*.

Other *in vivo* survival signals from the extracellular environment may also act in concert with *bcl-2*, contributing to drug resistance and promoting long-term cell survival. Ligation of CD40 on B lymphocytes in germinal centers can suppress apoptosis induced by calcium ionophore or anti-IgM *in vitro*. Combination of *bcl-2* expression and the provision of a culture environment that mimicked that of the germinal cen-

ters [CD40 ligation and the presence of interleukin-4 (IL-4)] increased resistance to apoptosis induced by the DNA-alkylating drug chlorambucil (CMB) in a Burkitt's lymphoma (BL) cell line transfected with either human *bcl-2* (BL-*bcl-2*) or a control plasmid (BL-Sv2) (Walker *et al.*, 1997). Ninety-six hours after the addition of 20 μ M CMB, 78% of the BL-Sv2 cells were apoptotic, whereas ligation of CD40 in BL-Sv2 cells reduced the proportion of apoptotic cells to 38%. Overexpression of *bcl-2* in BL-*bcl-2* cells reduced apoptosis to 41%. However, when the BL-*bcl-2* cells were treated with CMB together with ligation of CD40, apoptosis was reduced further to only 17% at 96 h (Walker *et al.*, 1997). These data demonstrate that drug-induced apoptosis can be modulated by *bcl-2* as well as by IL-4 and CD40 ligation. These factors appear to act together to enhance the proliferative potential after DNA damage. These findings have important implications regarding the development of drug resistance in B-cell lymphomas and future strategies for more effective therapy.

A partner protein of *bcl-2*, *bax* has an extensive amino-acid homology with *bcl-2* and is a promoter of apoptosis. Apparently, the equilibrium of *bcl-2* and *bax* heterodimers and homodimers and hence the ratio of *bcl-2* to *bax* are important for the susceptibility of cells to apoptosis-inducing stimuli. A significant correlation between the *bcl-2*-to-*bax* expression ratio and clinical response to chemotherapy ($P < 0.005$) was observed in AML when bone marrow samples obtained from 14 AML patients were examined using an immunophosphatase staining method (Stoetzer *et al.*, 1996). No patients with a *bcl-2*/*bax* ratio > 1.0 had a complete remission after induction therapy. Overall, these data provide further evidence for the existence of cross-talk between *bcl-2* and *bax* and suggest that these genes are important determinants of drug-induced apoptosis, thereby modulating resistance to chemotherapy.

The above findings suggest that new cancer treatments should take into account the expression of *bcl-2*, and novel therapeutics targeted against *bcl-2* may provide improved treatment of cancer by affecting physiological pathways distal to the targets of cytotoxic drugs.

Summary and Future Directions

A growing number of oncogenes has been shown to exert direct or indirect influence on cancer-cell

drug resistance, either functioning alone, in cooperation with other oncogenes, or in conjunction with classical drug-resistance genes, hormones, or other survival signals from the extracellular environment. These oncogenes may be used in the future as prognostic, predictive, or responsive factors for drug responses in clinical and laboratory investigations. Identification of oncogenes that can predict response to therapy is a very important issue because of the high cost of the treatments and their often uncomfortable side effects. Identification of oncogenes that are involved in drug resistance also provides an additional mechanism for non-*mdr* types of drug resistance. For example, even though the drugs cisplatin, methotrexate, and 5-Fluorouracil are not susceptible to the effects of the MDR-1 gene product, it is obvious from the above discussion that certain oncogenes can confer resistance to these drugs to cancer cells.

It is notable that controversial findings exist regarding the role of certain oncogenes in drug resistance. This raises the possibility that the role of oncogenes in drug resistance is far more complex than we can imagine, as it may be cell-, tissue-, tumor-, drug-, oncogene-, species-, timing-, sex-, or age-specific. Because most of the findings discussed above were obtained from two-dimensional *in vitro* cell-culture experiments, more comprehensive studies on the role of oncogenes in drug resistance using three-dimensional cell-culture (Theodorescu *et al.*, 1993) and *in vivo* animal models are desirable. Most importantly, large-scale clinical investigations are critical in determining the effects of oncogenes, alone or in cooperation with other oncogenes or factors, on drug resistance.

It is imperative to understand the molecular mechanisms underlying oncogene-mediated drug resistance for the purpose of conquering it. Certain oncogenes may regulate classical drug-resistance genes (e.g. *mdr*, *MRP*, *GST*, etc.) (Kopnin *et al.*, 1995), some may block drug-induced apoptosis, some may regulate signal transduction pathways that may impinge on drug function, some may target a DNA-repair pathway, and some may interact with hormones to protect cells from drug-induced toxicity. It is possible that each oncogene may exert drug-resistance function through multiple pathways or mechanisms.

Despite the remarkable progress made in understanding the role of oncogenes in drug resistance, the work so far has had little impact in the clinic. Scientists and clinicians in the drug resistance field need to further explore the potential of using this

knowledge to design new strategies to target oncogenes and sensitize cancer cells to therapy. Examples include the use of inhibitors of farnesyl transferase for *ras*-mediated drug resistance (Sepp-Lorenzino *et al.*, 1996), anti-EGFR antibodies for EGFR-mediated chemoresistance (Fan *et al.*, 1993), and the tyrosine kinase inhibitors tyrphostin AG825 and emodin to preferentially inhibit the *c-erbB2* kinase and to sensitize *c-erbB2*-overexpressing non-small cell lung carcinoma cells to chemotherapeutic agents (Tsai *et al.*, 1996; Zhang and Hung, 1996). It is anticipated that research on the role of oncogenes in drug resistance will present opportunities for new approaches to enhance the cytotoxicity of the standard chemotherapeutic agents that are currently used for human cancers. It is possible that enhanced antitumor activity can be achieved with maximum tolerated doses of drugs accompanied by functional perturbation of resistance-inducing oncogenes.

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