Epidermal Growth Factor Receptor Family and Chemosensitization

John Mendelsohn, Zhen Fan*

Alteration in the expression and activation of protein tyrosine kinases and cell cycle regulatory genes not only can lead directly to the perturbation of growth regulation but also may affect the sensitivity of cancer cells to conventional chemotherapy and radiation therapy. In recent years, numerous studies have explored the effects on chemosensitivity resulting from altered expression and activation of the epidermal growth factor (EGF) receptor and the HER-2/neu receptor.

In this issue of the Journal, Dixit et al. (1) report that reduced expression of EGF receptors achieved by introduction of EGF receptor antisense oligonucleotides is associated with increased resistance to cisplatin in MDA-468 human breast cancer cells, which express high levels of EGF receptors (>10⁵/cell). This finding was attributed to abrogation of cisplatin-induced apoptosis in the transfectants. Dixit et al. suggest that critical levels of EGF receptor signaling are necessary for apoptosis induced by cisplatin. A similar effect was not seen with other chemotherapeutic agents tested. While the data appear convincing, the conclusion seems to conflict with the view that human breast cancer cell lines selected for multidrug resistance frequently display increased EGF receptors or HER-2/neu receptors (2-4). In addition, experimental elevation of EGF receptor levels in the human breast cancer cell line ZR75B, which normally expresses low receptor levels, led to increased resistance to several cytotoxic chemotherapeutic drugs, including cisplatin (5).

An early study with an EGF receptor-blocking agent, monoclonal antibody (MAb) 108, showed that concurrent treatment with cisplatin at the time of KB carcinoma cell implantation resulted in enhanced antitumor activity (6). We have reported the curative treatment of well-established A431 squamous cell carcinoma xenografts with cisplatin followed by anti-EGF receptor MAb 225, which blocks activation of receptor tyrosine kinase and induces receptor down-regulation (i.e., reduction) (7). A possible mechanism was suggested by the preliminary finding that the repair efficiency of a cisplatin-damaged plasmid vector that encoded a luciferase reporter gene (pCMVLuc) was reduced in A431 cells exposed to MAb 225 (8). Antitumor activity was similarly enhanced when xenografts of A431 cells or MDA-468 cells were treated with doxorubicin in combination with MAb 225 (9) and when xenografts of MDA-468 cells were treated with paclitaxel in combination with MAb 225 (Baselga J, Kim YM, Mendelsohn J: unpublished observations). MAb 225 also enhanced the cytotoxicity of radiation therapy in A431 cells (10). Further studies with a number of cell lines have shown that treatment with MAb 225 causes a rise in the level of p27Kip1, accompanied by inhibition of cyclin-dependent kinase-2 (CDK2) and cell cycle arrest in the G₁ phase (11, 12). A phase I clinical trial with MAb 225 demonstrated tumor localization and lack of toxicity (13), and a human–mouse chimeric version of MAb 225 is presently in clinical trials in combination with chemotherapy.

Comparable studies have explored the results of altered expression and activation of the closely related HER-2/neu receptor. Treatment of human breast and ovarian cancer cell lines with cisplatin plus TAb 250 or 4D5 anti-HER-2/neu receptor MAb4D5, which can block cell proliferation, resulted in enhanced inhibition of cell growth in culture and in xenografts (14-16). In the case of MAb 4D5, this enhanced inhibition was associated with a reduction in the repair of cisplatin–DNA adducts in cultured ovarian cancer cells (16). Experimentally induced enhancement of HER-2/neu receptor expression in the human breast cancer cell line MDA-435, which normally expresses low receptor levels, conferred increased resistance to paclitaxel via mdr-1 gene-independent mechanisms (17). Chemosensitivity to cisplatin, doxorubicin, or etoposide also could be enhanced by exposure of cultures of non-small-cell lung carcinoma cell lines to tyrphostin AG825, a selective inhibitor of the HER-2/neu receptor tyrosine kinase. The enhanced drug sensitivity was observed in cell lines with high receptor levels but not in those with low receptor levels (18). Thus, there is ample experimental evidence that the sensitivity of tumor cells to cisplatin and other chemotherapeutic agents may be enhanced by antibodies and inhibitors that act on receptors in the EGF and HER-2/neu receptor family to block cell proliferation. The importance of this observation is emphasized by the results of a recent phase I clinical trial demonstrating that treatment of breast cancer patients with MAb 4D5 against HER-2/neu receptor induced objective partial responses in 10% of patients as well as one complete response, which provides evidence that anti-receptor MAb therapy can produce responses in human cancer (19). A phase II clinical trial with MAb 4D5 plus chemotherapy has been completed in patients with advanced breast cancer (20), and a phase III trial is under way.

These results appear to be different from those of Dixit et al. (1) suggesting that a reduction in EGF receptors causes reduced sensitivity to cisplatin. However, their observations are consistent with several reports showing that treatment of cultures with EGF, thereby activating EGF receptors, could enhance the sensitivity of an ovarian cancer cell line and other cancer cell lines to several chemotherapeutic agents (21-24) or to radiation therapy (25). Sensitization to chemotherapy induced by EGF was found to be independent of the mitogenic or anti-mitogenic

*Affiliation of authors: Department of Clinical Investigation, The University of Texas M. D. Anderson Cancer Center, Houston.

Correspondence to: John Mendelsohn, M.D., Box 91, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030-4095.

See “Notes” following “References.”
effects of EGF on cell proliferation (21). In contrast, EGF reduced sensitivity to cisplatin in studies with another ovarian cancer cell line (26). Preliminary experiments have also suggested that EGF can induce increased sensitivity to doxorubicin in A431 cells, which may be related to down-regulation of mutated p53 protein levels in those cells (27). In MCF-7 breast carcinoma cells transfected with an inducible vector expressing hercugulin, which could activate HER-2/neu receptors through heterodimerization with EGF, HER3, or HER4 receptors, sensitivity to doxorubicin was increased as a result of expression of hercugulin and this increased sensitivity was accompanied by up-regulation (i.e., increase) of topoisomerase II (28).

These studies lead to two conclusions: 1) Alteration of signal transduction pathways in the EGF/HER-2/neu receptor family of receptors can influence the sensitivity of a number of cell lines to cisplatin and other chemotherapeutic agents in culture and, in some cases, in xenograft models; and 2) the mechanisms explaining these effects have yet to be determined, which is emphasized by the fact that both activation and blockade of the receptor-signaling pathway can have similar effects in different experimental systems. The dilemma is reminiscent of the current paradox that tumor cells with altered p53 may show either enhanced or reduced chemosensitivity, again in a variety of different experimental systems (29-31).

The most important caveat is that the effects may be specific to particular tumor cell lines or cell types or to particular drugs. The mechanism of cell killing by chemotherapeutic agents is considered to be a common pathway involving activation of programmed cell death (apoptosis). Large numbers of molecules in cells are known to regulate the apoptotic event in response to cytotoxic agents; these molecules include the p53 protein, which is mutated in the majority of human cancers that have been examined. Bcl-2, BAX, and related molecules are also regulatory (32). In an early stage of DNA damage-induced, p53-independent apoptosis, retinoblastoma (Rb) protein appears to be the target of an interleukin 1β-converting enzyme-like (ICE) protease that can initiate apoptosis, suggesting a role for Rb in regulating cell death (33-35). The products of other cell cycle regulatory genes such as the cyclin-dependent kinase inhibitors p16 (36), p21 (37), and p27 (38) and overexpression of cyclin D (39) have also been reported to be associated with resistance to certain drugs. The status of these and other molecules in the various tumor cell lines studied may have had an important influence on the response to receptor blockade and to the damaging effects of different chemotherapeutic agents. For example, in the MDA-468 cells studied by Dixit et al. (1), it is known that the Rb gene is deleted and the p53 gene is mutated (40,41). In these particular cells, an increase or decrease in signaling from EGF receptors could have effects different from those observed in other cell lines. In another example, we have observed that blockade of EGF receptors by MAb 225 in DiFi colon carcinoma cells results in apoptosis (in contrast to the growth arrest seen in most cells) (42); subsequent studies showed very low levels of Bcl-2 in these cells, and experimental elevation of Bcl-2 protein in DiFi cells by introducing a vector carrying Bcl-2 gene suppressed the apoptotic response to MAb 225 (Kumar R; personal communication). In recognition of the importance of knowing the status of essential cell-regulatory molecules for understanding the effects of cytotoxic agents, investigators at the National Cancer Institute, Bethesda, MD, are performing a massive screening effort to provide detailed molecular characterization of 60 human tumor cell lines, for which extensive data on susceptibility to the entire menu of known chemotherapeutic agents are available (43).

In the case of cisplatin, timing of receptor manipulation and exposure to chemotherapy may be critical. Our preliminary data from studies with A431 xenografts (Fan Z, Lu Y, Mendelsohn J: unpublished observations) suggest that EGF receptor blockade before drug exposure is less effective than after drug exposure for chemosensitization. It is quite possible that, in some cell lines, receptor activation could potentiate drug-induced cell damage, whereas subsequent receptor blockade could interfere with repair of the damage.

The interactions between chemotherapeutic agents and various molecular steps in the signal transduction pathways that regulate cell proliferation are under intensive study. Examples, in addition to those briefly discussed above, include inhibitors of farnesyl transferase (44) and protein kinase C (45). We anticipate that research on these interactions will present opportunities for new approaches to enhancing the cytotoxicity of the standard chemotherapeutic agents that we are using to treat human cancer today: Instead of increasing drug doses to supertoxic levels and obviating some toxic effects by providing hematopoietic rescue (stem cell transfusion and colony-stimulating factor stimulation), it is possible that enhanced antitumor activity can be achieved with maximum tolerated doses of drugs accompanied by perturbation of a signal transduction pathway. Why should this new strategy be relatively selective for malignant cells compared with normal cells? Because of mutations in p53 and other molecules, malignant cells often disobey checkpoint signals that regulate cell proliferation. This situation could render the cells more susceptible to irreparable damage, if they simultaneously ignore two separate checkpoints activated both by alteration in growth regulatory signals and by concurrent drug-induced damage to DNA or other vital molecules.

References

7. Fan Z, Basela, Masui H, Mendelsohn J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus cis-


Notes
Supported by Public Health Service grants CA42060, CA37641, CA65746, and CA68425 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
Dr. Mendelsohn has a financial interest in ImClone Systems, Inc., which is performing clinical trials with human–mouse chimeric monoclonal antibody 225.