On Receptor Inhibitors and Chemotherapy

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In the search for rational targets for drug development in cancer, receptor tyrosine kinases have become a favorite. The rationale for directing efforts to developing inhibitors of these receptors is compelling. Mutated or overexpressed growth factor receptors play a pivotal role in malignant transformation and in maintaining the growth of cancers in model systems. Strategies that inactivate growth factor receptors or their downstream pathways halt tumor growth. Of particular recent interest are members of the EGFR family including the EGFR (c-erbB1/HER1) and Her2 (c-erbB2/HER2) molecules. Both receptors are overexpressed in a broad range of human cancers of epidermal origin, and their presence has been associated with a poor prognosis and resistance to cytotoxic chemotherapy (1–4). These receptors are composed of an external domain responsible for ligand binding. Binding in turn stimulates dimerization and activates an internal domain that has tyrosine kinase activity. Both the receptor ligand binding function and the kinase domain present potential targets for inhibition of receptor function. In recent years, molecules of both types (external domain antagonists and kinase inhibitors) have reached the clinic and shown evidence of modest activity in breast cancer [Herceptin (5)] and non-small cell lung cancer [EGFR antibody C225 and Iressa (6, 7)].

Although these effects are important in demonstrating the potential of such agents, the ultimate value of the antireceptor approach may lie elsewhere, namely, in the combined use of these inhibitors with cytotoxic drugs or irradiation, a subject addressed in several recent articles in this journal (8–11) and in related publications (12–14).

The mechanism whereby tyrosine kinase inhibitors enhance the activity of cytotoxic drugs is complex and incompletely understood. Growth factor signaling pathways provide a stimulus for proliferation through a complex receptor-to-nucleus cascade. In addition, they up-regulate the expression of genes that antagonize apoptosis, or programmed cell death, such as Bcl-2 and Bcl-XL and thereby raise the threshold for cell death in response to DNA damage (15). Finally, these pathways stimulate the secretion of angiogenic factors such as VEGF (16). All of these actions confer a growth or survival advantage, but the apoptotic effects seem most important in combination with drugs.

When combined with drugs, inhibitors of growth factor receptors enhance the efficacy of conventional cytotoxic agents. This has been demonstrated convincingly with Herceptin and paclitaxel or Herceptin and doxorubicin in xenograft experiments (17) and in patients with metastatic breast cancer (12), for whom the combination has resulted in a significant increase in response rates and improved survival. Promising preclinical experiments with C225 (18, 19), an anti-EGFR monoclonal antibody, have led to clinical trials of the antibody combined with radiotherapy in head and neck cancer (13, 14). Of nine patients with head and neck cancers treated with C225 and cisplatin, six had major responses, and three of these six had previously failed cisplatin-based chemotherapy (13). Fourteen of 15 patients achieved complete responses after treatment with C225 and once- or twice-daily irradiation for locally advanced head and neck malignancies. In these patients, a complete response rate of 50% or less would have been expected with radiation alone (14).

The explanation for these effects may lie in the effects of receptor inhibition on the threshold for apoptosis. In the case of paclitaxel interaction with Her2, paclitaxel disrupts microtubule integrity, leading to cell cycle arrest and apoptosis (20). Overexpression of Her2 confers resistance to paclitaxel by increasing levels of p21, a cyclin-dependent kinase inhibitor, which delays entrance into mitosis and prevents mitotic arrest (20). Resistance to paclitaxel can be overcome by blocking the Her2 receptor-mediated signaling, and the consequent increase in p21. A second antiapoptotic signal emanates from the EGFR, which, on ligand binding, activates a number of downstream signals (21–24) including protein kinase A, which, in turn, phosphorylates bcl-2 (22). This interaction provided the rationale for combined therapy with C225, antisense oligonucleotides targeting the RIa regulatory subunit of protein kinase A, and docetaxel in the human breast cancer cell line ZR-75-1 (24). These three agents, used in combination at suboptimal doses, led to a greater degree of Bcl-2 phosphorylation and apoptosis than seen with any single agent alone in this cell line (24).

Receptor blockade with ZD1839, a low molecular weight inhibitor of EGFR, exerts synergistic effects with paclitaxel or other chemotherapy in xenograft experiments (25). ZD1839, an anilinoquinazoline, is a potent and selective inhibitor of EGFR receptor kinase in vitro and in vivo. It has strong antiproliferative effects against the growth of the A431 tumor, which has high levels of expression of EGFR (26). Sirotinak et al. (8) report in this issue of Clinical Cancer Research that the combination of ZD1839 with taxanes, platinum, or the folate antagonist edatrexate markedly enhances antitumor activity. The combination of ZD1839 with all cytotoxic agents required a 2-fold or greater attenuation of the ZD1839 dose below its single-agent maximum dose of 150 mg/kg. Interestingly, the degree of en-
hancement of cytotoxic activity was not dependent on high levels of EGFR expression. As a single agent at the maximum tolerated dose, ZD1839 induced partial regression of A431, a vulvar tumor expressing high levels of EGFR. ZD1839 also demonstrated 50–55% growth inhibition against the LX-1 lung tumor, which expresses very low levels of EGFR. A related compound, CP-358774, inhibited epidermal growth factor-dependent cell proliferation in a head and neck squamous cell carcinoma model with drug concentrations in the nanomolar range and eliminated in vitro epidermal growth factor-induced autophosphorylation of tumor (27). As receptor inhibitors, small molecules have important potential advantages compared with monoclonal antibodies; they should have better tissue penetration and potential oral bioavailability and, finally, an ability to inhibit the intracellular receptor tyrosine kinase activity directly, rather than as a secondary effect of interaction with the external receptor domain. They may have the important limitations of potential pharmacokinetic interaction with other agents, susceptibility to drug transporters such as MRP and MDR, and promiscuous interaction with related tyrosine kinases. Like the monoclonal antibodies, the small molecule receptor inhibitors may be tumoricidal rather than tumoricidal; it may be difficult to appreciate their primary activity as single agents. Imaging for metabolic effects or repetitive tumor biopsies may be helpful in establishing activity in humans.

Studies by Inoue et al. (9) and Perrotte et al. (16) also address the effects of receptor inhibitors on angiogenesis, a process that may also contribute to the synergy between receptor inhibitors and chemotherapy. Perrotte et al. (16) reported that in vitro treatment with C225 inhibited mRNA and protein production of VEGF, IL-8, and bFGF by the highly metastatic human TCC 253JB-V cells in a dose-dependent manner. C225 therapy of nude mice with orthotopically implanted TCCs resulted in inhibition of growth and metastases compared with controls. The expression of protein VEGF, IL-8, and bFGF as determined by immunohistochemical staining was significantly lower in treated tumors as compared with the expression of these proteins in controls. Microvessel density was significantly lower in tumors treated for 5 weeks with C225 as compared with the vessel density in control tumors. Furthermore, the down-regulation of VEGF, IL-8, and bFGF mRNA and protein preceded the reduction in microvessel density. Their data suggest that a mechanism that contributes, at least in part, to the antitumor effect of EGFR blockade therapy with C225 is inhibition of angiogenesis. C225 has also been shown to down-regulate VEGF expression in A431 cells (28) and in renal cell carcinoma cell lines (29). In human pancreatic carcinoma growing orthotopically in nude mice, systemic therapy with C225 alone and with C225 in combination with gemcitabine resulted in growth inhibition, tumor regression, and abrogation of metastases (10). This was associated with a significant reduction in PCNA-positive cells, production of VEGF and IL-8, and microvessel density. Futhermore, Inoue et al. (9) evaluated the efficacy of using the combination of C225 and paclitaxel. Paclitaxel has known antitumor effects against TCC of the bladder, as a single agent and in combination with other cytotoxic agents. The combination of C225 (at the IC_{50} dose of 100 µg/ml) and paclitaxel (at the IC_{50} dose of 10 µg/ml) resulted in increased apoptosis in vitro as compared with either agent alone. In vivo, treatment with paclitaxel, followed by C225, led to significantly greater regression of tumors compared with treatment with either agent alone. When measured by immunohistochemistry for PCNA and terminal deoxynucleotidyl transferase-mediated nick end labeling, the number of PCNA-positive cancer cells was significantly lower in tumors from mice treated with the combination of C225 and paclitaxel, compared with either agent alone. Therapy with initial paclitaxel followed by C225 significantly increased the number of apoptotic cancer cells; this treatment also resulted in an increase in the apoptotic index for endothelial cells. Paclitaxel did not enhance the reduction of microvessel density seen after C225 therapy alone. The authors speculate that the increased antitumor activity with combined activity is the result of the summation of effects on tumor cell pathways regulating apoptosis.

The combination of conventional chemotherapy agents with inhibitors of the receptor tyrosine kinase pathways has exciting potential for clinical use. Clinical trials combining C225 with chemotherapy or radiation for head and neck cancers are ongoing. Clinical testing with ZD1839 is also currently under way in patients with metastatic lung and breast cancer. Because the EGFR is frequently overexpressed in epithelial tumors and has been correlated with tumor resistance to cytotoxic agents and chemotherapy, the emerging preclinical data are particularly encouraging and warrant further study. Clinical investigators must appreciate that the value of these new, targeted drugs may not be obvious in single-agent studies but may only be realized in combination trials with chemotherapy. The early initiation of combination studies is an important strategy in the age of “molecularly targeted” drug discovery and development.

References


