Enhanced Sensitivity to the HER1/Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Erlotinib Hydrochloride in Chemotherapy-Resistant Tumor Cell Lines

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ABSTRACT

Purpose: Erlotinib (Tarceva, OSI-774) is a potent and specific inhibitor of the HER1/epidermal growth factor receptor (EGFR) tyrosine kinase. In phase II clinical studies, oral erlotinib monotherapy has shown antitumor activity in patients with advanced non–small cell lung cancer, head and neck cancer, and ovarian cancer after the failure of standard chemotherapy. We hypothesized that some tumors treated with multiple cytotoxic therapies may become more dependent on the HER1/EGFR signaling pathways for survival.

Experimental Design: The growth-inhibitory effect of erlotinib was tested on 10 pairs of chemosensitive, parental, and chemoresistant tumor cell lines.

Results: Enhanced sensitivity to erlotinib was observed in the doxorubicin-resistant human breast cancer cell line MCF-7, paclitaxel-resistant human ovarian carcinoma cell line A2780, and cisplatin-resistant human cervical carcinoma cell line ME180. The IC50 values of erlotinib in the resistant cell lines were 2- to 20-fold lower than those in the corresponding parental cell lines. This enhanced sensitivity to erlotinib correlated with higher HER1/EGFR and phospho-HER1/EGFR expression when compared with the corresponding parental cell lines. Acquired resistance to cytotoxic agents was not associated with cross-resistance to erlotinib. AE-ME180/CDDP-resistant xenografts showed greater sensitivity to erlotinib than parental ME180 xenografts did.

Conclusions: Our findings suggest that acquired resistance to cytotoxic therapy in some tumors is associated with enhanced sensitivity to HER1/EGFR inhibitors, which correlates with increased HER1/EGFR expression. These data may explain some of the observed clinical activity of HER1/EGFR inhibitors in patients previously treated with multiple therapies. HER1/EGFR tyrosine kinase inhibitors may be more effective as second- or third-line treatment for certain patients with tumors that were previously treated with multiple chemotherapy regimens.

INTRODUCTION

HER1/epidermal growth factor receptor (EGFR) is dysregulated in many human malignancies and is a potential target for therapeutic intervention (1–4). The HER1/EGFR signaling pathway plays a pivotal role in controlling tumor growth and progression, apoptosis, and angiogenesis, and may impair the response of tumor cells to cytotoxic agents. In some tumor types, dysregulation of HER1/EGFR has been associated with chemoresistance and poor prognosis (5–9). Both HER1/EGFR-receptor-blocking antibodies (e.g., cetuximab and ABX-EGF) and small-molecule inhibitors of tyrosine kinase (e.g., erlotinib and gefitinib) have been explored as novel approaches in the treatment of those malignancies refractory to conventional chemotherapy.

Erlotinib HCl (erlotinib, Tarceva; OSI-774) is one of the HER1/EGFR tyrosine-kinase inhibitors (TKI) currently under extensive investigation. It is a high-affinity, potent, reversible, and specific HER1/EGFR TKI, which competes with ATP for its binding site on the intracellular domain of the receptor. Preclinical studies have shown that very low concentrations are required to inhibit activity against isolated tyrosine kinase (IC50, 2 nmol/L), to reduce HER1/EGFR autophosphorylation in intact human tumor cells in vitro (IC50, 20 nmol/L), and to inhibit the EGF-dependent proliferation of cells (10). It acts by inducing the expression of the cell-cycle inhibitor p27, and suppressing the expression of the cell-cycle promoter cyclin D1, thereby blocking cell-cycle progression at the G1 phase (10). Treatment of mice bearing human HNS5 head and neck carcinoma xenografts with erlotinib profoundly inhibited tumor growth (11). More than 70% reduction in HER1/EGFR autophosphorylation was found in HNS5 xenografts treated with erlotinib (11). These studies established a direct relationship between HER1/EGFR inhibition and inhibition of tumor growth. Phase I clinical trials of erlotinib in patients with advanced refractory solid tumors have shown good tolerability, with mild to moderate cutaneous rash and diarrhea as the main side effects (12, 13).

Three phase II clinical trials have shown antitumor activity of erlotinib in patients with advanced non–small cell lung cancer, squamous-cell carcinoma of the head and neck, and ovarian carcinoma (14–16). The patients enrolled in the non–small cell lung cancer and ovarian cancer studies all had advanced tumors that expressed HER1/EGFR and were refractory to multiple prior chemotherapy regimens. Subset analysis of the non–small cell lung cancer phase II study suggested that responses to erlotinib were independent of the...
number of prior regimens (14). The phase II erlotinib data support HER1/EGFR inhibitors as therapy against tumors previously treated with multiple cytotoxic agents. Gefitinib has also shown efficacy in clinical studies in patients previously treated with multiple cytotoxic agents (IDEAL), but was not effective in patients who have not received chemotherapy (INTACT; refs. 17–20). These data suggest that some tumors treated with cytotoxic agents may become more dependent on HER1/EGFR signaling pathways for survival and, therefore, may become more sensitive to HER1/EGFR inhibitors. To explore this hypothesis, we compared the antitumor activity of erlotinib on chemosensitive cell lines with matched chemoresistant sublines derived from those cells.

MATERIALS AND METHODS

Cell Lines. A panel of human cancer cell lines in their parental and chemoresistant sublines was used in our study. The tissue origin and reference information are shown in Table 1. The AE-ME180/CDDP cells were developed in our laboratory by continuous exposure of ME180 cells to CDDP. All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO2 and 95% air.

Drugs and Chemicals. Erlotinib [6,7-bis(2-methoxyethoxy)-quinazolin-4-yl-(3-ethynylphenyl)amine; MF = C22H23N3O4] was kindly provided by OSI Pharmaceuticals (Albert Einstein College of Medicine, Bronx, NY). All dosage forms were freshly prepared prior to each experiment.

Cell Growth Assays. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-tetrazolium dye assay was used to evaluate the growth-inhibitory activity of a drug at various concentrations. One hundred and thirty-five microliters of an exponentially growing cell suspension (5 × 104 cells/mL) was used to evaluate the growth-inhibitory activity of a drug at various concentrations. One hundred and thirty-five microliters of 5 mg/mL MTT solution was added and the cells further incubated for 2 hours. The MTT formazan was solubilized in 100 μL DMSO as a stock solution, and then further diluted to desired concentrations for in vitro experiments, or prepared for p.o. administration as described (11). Doxorubicin was purchased from Adria Laboratories (Columbus, OH). Cisplatin and paclitaxel were obtained from Sigma Chemical Co. (St. Louis, MO). Epothilone B was kindly provided by Dr. Susan B. Horwitz (Albert Einstein College of Medicine, Bronx, NY). All dosage forms were freshly prepared prior to each experiment.

Cell Growth Assays. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-tetrazolium dye assay was used to evaluate the growth-inhibitory activity of a drug at various concentrations. One hundred and thirty-five microliters of an exponentially growing cell suspension (5 × 104 cells/mL) were seeded into a 96-well plate overnight and then each drug added and the cells further incubated for 2 hours. The MTT

RESULTS

In vivo Tumor Growth Inhibition Studies. ME180 and AE-ME180/CDDP cells were harvested during exponential growth of the cell culture. Five athymic mice were randomized in each group prior to inoculation and 2 × 106 parental ME180 or AE-ME180/CDDP cells were implanted s.c. in the flank of each mouse. After tumor became palpable (2–4 mm diameter) on day 6 post-inoculation, mice in the treatment group were given 100 mg/kg erlotinib orally (oral gavage) daily Monday to Friday for 4 weeks. Tumors were measured across two dimensions, and the tumor volume (mm3) was calculated using the formula: tumor volume = (length × width2)/2, according to standard methods (22).

RESULTS

In vitro Growth-Inhibitory Effect of Erlotinib on Chemosensitive and Chemoresistant Tumor Cell Lines. To assess altered sensitivity to erlotinib, the growth-inhibitory effects of erlotinib on chemosensitive parental cell lines and matched chemoresistant sublines were compared. Each pair of cell lines was treated with a specific chemotherapy agent or erlotinib at various concentrations for 72 hours before MTT

Table 1 Parental tumor cell lines and their chemoresistant sublines

<table>
<thead>
<tr>
<th>Parental cell line</th>
<th>Tumor of origin</th>
<th>Resistant subline</th>
<th>Drug resistant to</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME180</td>
<td>Cervical Cancer</td>
<td>AE-ME180/CDDP</td>
<td>Cisplatin</td>
<td>see Materials and Methods; Ling et al. (29)</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Breast Cancer</td>
<td>MCF-7/Dox</td>
<td>Doxorubicin</td>
<td>Perez-Soler et al. (30)</td>
</tr>
<tr>
<td>A2780</td>
<td>Ovarian Cancer</td>
<td>A2780/Taxol</td>
<td>Paclitaxol</td>
<td>Giannakakou et al. (31)</td>
</tr>
<tr>
<td>KB</td>
<td>Head and Neck Cancer</td>
<td>KB/VBL</td>
<td>Vinblastin</td>
<td>Cornwell et al. (33)</td>
</tr>
<tr>
<td>HT212/9</td>
<td>Cervical Cancer</td>
<td>HT212/1/CDDP</td>
<td>Cisplatin</td>
<td>Britten et al. (34)</td>
</tr>
<tr>
<td>HT180/8</td>
<td>Cervical Cancer</td>
<td>HT180/1/CDDP</td>
<td>Cisplatin</td>
<td>Britten RA et al. (34)</td>
</tr>
<tr>
<td>A549</td>
<td>Lung Cancer</td>
<td>A549/Taxol</td>
<td>Paclitaxol</td>
<td>Laura et al. (35)</td>
</tr>
<tr>
<td>A549</td>
<td>Lung Cancer</td>
<td>A549/Epothilone</td>
<td>Epothilone</td>
<td>He et al. (36)</td>
</tr>
<tr>
<td>LoVo</td>
<td>Colon Cancer</td>
<td>LoVo/CDDP</td>
<td>Cisplatin</td>
<td>Yang et al. (37, 38)</td>
</tr>
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The growth-inhibitory activity of each drug was determined by its IC50 value. Table 2 shows that high chemoresistance indices were found in all resistant cell lines. IC50 values of chemotherapy agents were significantly higher in the sublines than in the parental lines, confirming that each subline was resistant to the selected chemotherapy agent.

In parallel studies, the same panel of cell lines was exposed to erlotinib. Enhanced sensitivity to erlotinib was observed in three chemoresistant sublines: AE-ME180/CDDP, MCF-7/Dox, and A2780/Taxol. The IC50 values of erlotinib in these resistant cell lines are 3- to 20-fold lower than their parental cell lines (P < 0.01 in AE180/CDDP and MCF-7/Dox, P < 0.05 in A2780/Taxol). Erlotinib resistance indices remained close to 1 in other resistant sublines A2780/CDDP, KB/VBL, HT212/11/CDDP, and A549/Epothilone, demonstrating that acquired resistance to cytotoxic agents is not associated with cross-resistance to erlotinib in these chemoresistant cell lines. Three pairs of tumor cell lines, A549 and A549/Taxol, LoVo and LoVo/CDDP, and HT180/8 and HT180/1/CDDP, did not reach 50% growth reduction after exposure to up to 20 μmol/L erlotinib.

The most dramatic increase in sensitization was seen in AE-ME180/CDDP. This chemoresistant subline had a more than 20-fold greater sensitivity to erlotinib than its parental cell line (resistance index < 0.05). The ME180 parental line was sensitive to cisplatin but resistant to erlotinib (IC50, 1.53 ± 0.93 versus >20 μmol/L), whereas AE-ME180/CDDP was much more sensitive to erlotinib (IC50, 1 μmol/L).

HER1/EGFR Expression in Parental and Chemoresistant Cell Lines. To investigate the molecular basis for the increased sensitivity of chemoresistant tumors to erlotinib, we used Western blot analysis to determine the level of HER1/EGFR expression in parental chemosensitive and chemoresistant variant cell lines (Fig. 1A). Higher HER1/EGFR expression was found in seven chemoresistant cell lines (MCF-7/DOX, ME180/CDDP, A2780/Taxol, A549/EPO, A549/Taxol, HT212/11/CDDP, and HT180/1/CDDP) compared with the corresponding parental cell lines. Lower EGFR expression was found in three chemoresistant sublines (i.e., A2780/CDDP, KB/VBL, and LoVo/CDDP). Among the seven variants expressing higher HER1/EGFR, three cell lines (i.e., AE-ME180/CDDP, MCF-7/Dox, and A2780/Taxol) have shown an association between increased HER1/EGFR expression and increased erlotinib sensitivity, suggesting that higher HER1/EGFR expression may be necessary for enhanced sensitivity to HER1/EGFR inhibitors. In these three resistant cell lines, expression of both total and phosphorylated HER1/EGFR was higher than in their parental cell lines (Fig. 1B), suggestive of receptor activation and thereby providing a good target for the HER1/EGFR inhibitor. It was also noted that four chemoresistant cell lines (i.e., A549/EPO, A549/Taxol, HT212/11/CDDP, and HT180/1/CDDP) did not show enhanced sensitivity to erlotinib, despite elevated levels of HER1/EGFR expression. In these chemoresistant cell lines, higher basal levels of EGFR phosphorylation were not observed (data not shown). This suggests that HER1/EGFR expression may not be sufficient for acquiring increased tumor sensitivity to HER1/EGFR inhibitors, and that receptor kinase activity is important for responsiveness to erlotinib.

Table 2 In vitro growth-inhibitory effect of erlotinib on parental and chemoresistant cell lines

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IC50/Chemo (μmol/L)</th>
<th>RI</th>
<th>IC50/Erlotinib (μmol/L)</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME180</td>
<td>1.53 ± 0.93</td>
<td>9.3</td>
<td>0.94 ± 0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>AE-ME180/CDDP</td>
<td>14.30 ± 0.47</td>
<td>20</td>
<td>0.94 ± 0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>MCF-7</td>
<td>0.43 ± 0.21</td>
<td>&gt;20</td>
<td>0.94 ± 0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>MCF-7/Dox</td>
<td>&gt;17.2 &gt;40</td>
<td>0.24</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A2780</td>
<td>0.65 ± 0.20</td>
<td>0.24</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A2780/Taxol</td>
<td>9.75 ± 1.12</td>
<td>0.28</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A2780/CDDP</td>
<td>1.5</td>
<td>18.0</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A2780/CDDP</td>
<td>22.3</td>
<td>18.0</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>KB/VBL</td>
<td>&lt;0.001</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
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<tr>
<td>HT212/9/CDDP</td>
<td>0.67</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
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<tr>
<td>HT212/11/CDDP</td>
<td>3.0</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
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<tr>
<td>HT180/8/CDDP</td>
<td>1.43 ± 0.63</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>HT180/1/CDDP</td>
<td>13.67 ± 1.5</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A549</td>
<td>1.6 ± 1.42</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A549/Taxol</td>
<td>&gt;11.71</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A549</td>
<td>0.01</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>A549</td>
<td>2.20</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Epothilone</td>
<td>20.33</td>
<td>18.0</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>LoVo/CDDP</td>
<td>&gt;33.33</td>
<td>&gt;20</td>
<td>15.40 ± 1.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

NOTE: IC50 is the drug concentration needed for 50% growth reduction on the survival curve. Each value is the mean ± SD of three or four independent experiments, or the average value of two independent experiments. Resistance index (RI) is the ratio between IC50 of drug in chemoresistant cell line and IC50 of drug in parental cell line.

Effect of Erlotinib on Mitogen-Activated Protein Kinase and Akt Activity. HER1/EGFR activates several cytoplasmic signal-transduction pathways. We investigated whether inhibition of mitogen-activated protein kinase (MAPK) or Akt activity may correlate with the enhanced antitumor effects of erlotinib in chemoresistant cell lines. Parental ME180 and AE-ME180/CDDP cells were treated with erlotinib at various concentrations and then stimulated with EGF (100 ng/mL). As seen in Fig. 2, erlotinib at 0.1 μmol/L markedly inhibited ERK1/2 activity in parental ME180, which did not occur to AE-ME180/CDDP. However, erlotinib at 1 μmol/L blocked ERK1/2 activity almost completely in both ME180 and AE-ME180/CDDP cells, and there was no significant difference in the degree of inhibition of the two cell lines. This result suggests that inhibition of MAPK may not be the molecular mechanism for increased sensitivity to erlotinib in the CDDP-resistant ME180 cell line.

HER1/EGFR kinase also stimulates the recruitment and activation of PI3 kinase at the cell membrane leading to activation of the cytosolic serine/threonine kinase Akt. It was found that erlotinib down-regulated Akt activity effectively in both ME180 and AE-ME180/CDDP cells (Fig. 2). These data suggest that enhanced sensitivity to erlotinib in the AE-ME180/CDDP cell line is probably mediated by downstream molecules and signaling pathways other than MAPK and Akt. Nevertheless, some differences should be pointed out. When comparing Fig. 2 with Table 3, we found that erlotinib (1 μmol/L) inhibits the growth of CDDP-resistant ME180 cells by 50% in vitro, which correlates with the erlotinib concentration required for
inhibition of MAPK and Akt activities. However, erlotinib (1 μmol/L) inhibits EGF-stimulated MAPK and Akt activities in the CDDP-sensitive, parental ME180 cell line, yet has no effects on cell growth at this concentration.

**In vivo Antitumor Effects of Erlotinib on ME180 CDDP-Sensitive and CDDP-Resistant Tumor Xenografts.** To examine the effect of erlotinib on the growth of chemosensitive and chemoresistant tumors in vivo, the antitumor activity of erlotinib was studied in mice bearing CDDP-sensitive and CDDP-resistant ME180 tumor xenografts. Table 2 illustrates that the AE-ME180/CDDP cell line had greater sensitivity to erlotinib than its parental cell line in an in vitro growth-inhibition assay. Athymic nude mice were inoculated with either parental ME180 or AE-ME180/CDDP cells. Mice in the treatment group received erlotinib at 100 mg/kg orally (oral gavage) daily from Monday to Friday for 4 weeks. Figure 3 shows mean tumor volumes on the days indicated post-inoculation. Erlotinib treatment had little effect on ME180 xenograft growth compared with no treatment (Fig. 3A). In contrast, erlotinib decreased the mean tumor volume of AE-ME180/CDDP xenografts (Fig. 3B). Similar to the results obtained in vitro, erlotinib shows enhanced antitumor activity in vivo against the CDDP-resistant ME180 tumor relative to the CDDP-sensitive parental ME180 tumor. Analysis of tumor xenografts using Western blotting showed a decrease in EGF-stimulated receptor phosphorylation in the CDDP-resistant ME180 tumor after treatment with erlotinib (data not shown).

**DISCUSSION**

This study suggests that, in some tumors, acquired resistance to cytotoxic agents is associated with increased HER1/EGFR expression and increased sensitivity to HER1/EGFR TKIs such as erlotinib. Increased HER1/EGFR expression may be a survival response by some tumors exposed to chemotherapeutic agents. Similar observations of increased HER1/EGFR expression in response to chemotherapeutic agents have been reported previously (23). However, the level of receptor expression does not always correlate with sensitivity to erlotinib. In our study, several chemoresistant cell lines with increased HER1/EGFR expression did not exhibit significant increased sensitivity to erlotinib relative to the parental cell line, suggesting that HER1/EGFR expression may be required, but not sufficient for increased tumor response to HER1/EGFR TKIs. In phase II studies in non–small cell lung cancer patients, HER1/EGFR was expressed to some degree on all tumors, however, not all of the patients derived clinical benefit from erlotinib (14). These preclinical and clinical observations suggest that determinants of sensitivity to HER1/EGFR-inhibitors

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**Fig. 1** Total HER1/EGFR expression and baseline phospho-HER1/EGFR activity in chemosensitive parental and chemoresistant variant cell lines. A, total HER1/EGFR expression determined by Western blot analysis. Thirty micrograms of total cellular lysates prepared from chemosensitive parental (W) and chemoresistant variant cells (R) were resolved by 7.5% SDS-PAGE, transferred to a nitrocellulose membrane, and blotted with anti-EGFR antibody as described in Materials and Methods. B, baseline phospho-EGFR activity in three pairs of chemosensitive parental (W) and chemoresistant resistant (R) sublines determined by Western blot analysis. The samples were resolved by 12% SDS-PAGE and anti-phospho EGFR antibody was used for immunoblotting as described in Materials and Methods.
are complex, involving the interplay of multiple factors such as receptor dimerization and various downstream signal-transduction pathways. In addition, interruption of a single signaling network or transforming molecule may not block tumor growth. Recently, Lynch et al. (24) reported that specific mutations in EGFR gene correlate with clinical responsiveness to the TKI gefitinib in a subgroup of patients with non–small cell lung cancer. Mutations were clustered around the ATP-binding pocket of the tyrosine kinase domain of EGFR, and were identified in eight of nine patients with a response to gefitinib. In vitro analyses, these mutations lead to enhanced tyrosine kinase activity in response to EGF and increased sensitivity to inhibition by gefitinib. These findings provide an important insight into underlying molecular mechanism for differential responsiveness to EGFR TKIs. It would be interesting to find out whether chemoresistant cell lines that respond to erlotinib have acquired activating mutations in the EGFR kinase.

Activation of HER1/EGFR by ligand-binding results in receptor dimerization, which subsequently activates the intracellular kinase domain resulting in receptor phosphorylation. Phosphorylation of HER1/EGFR is a critical first step in intracellular signaling. The degree of HER1/EGFR phosphorylation may enable us to identify tumor cells whose survival is more dependent on HER1/EGFR. Such tumor cells may be more likely to respond to HER1/EGFR TKIs, although it is uncertain whether the expression of HER1/EGFR correlates strongly with tumor responsiveness. HER1/EGFR functionality is closely linked to other HER family members by common ligands and cross-activation through receptor heterodimerization. Inhibition of HER1/EGFR activity can concomitantly lead to signal-transduction inhibition of other HER-family members. Differential expression of HER family members can also contribute to the varying responses to HER1/EGFR inhibitors (25). Dual inhibitors of HER1/EGFR may result in better efficacy.

Table 3  

<table>
<thead>
<tr>
<th></th>
<th>Parental ME180</th>
<th>AE-ME180/CDDP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Erlotinib</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50 values of erlotinib in parental and chemoresistant cell lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental ME180</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td>AE-ME180/CDDP</td>
<td>0.94 ± 0.24</td>
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</table>

Fig. 2  Effect of erlotinib on phosphorylation of MAPK (ERK1/2) and Akt in ME180 parental and AE-ME180/CDDP cells. Cells were incubated in medium containing the indicated concentrations of erlotinib for 2 hours. At the end of the 2-hour incubation, 100 ng/mL EGF was added for 5 minutes. Total cellular lysates were prepared and separated by 12% SDS-PAGE. After transferring to a nitrocellulose membrane, blots were probed with anti-phospho-ERK1/2, anti-ERK1/2, anti-phospho-Akt, or anti-Akt antibodies, respectively. Relative changes of pERK1/2 and pAkt levels were quantified by densitometric analysis and normalized to the densitometric value of basal pERK1/2 or basal pAkt (ratios as listed under each corresponding band). IC50 values of erlotinib in ME180 parental cells and AE-ME180/CDDP-resistant cells (see Table 3).
expressing tumor cell lines could be subdivided into two groups based on their sensitivity to HER1/EGFR TKIs. In the HER1/EGFR TKI-sensitive cell lines, receptor phosphorylation, MAPK activity inhibition, and growth inhibition were concordant, and occurred at low inhibitor concentrations. In the HER1/EGFR TKI-insensitive group, receptor inhibition occurred at low TKI concentrations, but growth inhibition required much higher concentrations of HER1/EGFR TKIs. These data suggest that HER1/EGFR TKIs inhibit the HER1/EGFR-ras/MAPK pathway, but this inhibition is not always associated with subsequent tumor growth inhibition.

In our studies, we evaluated matched pairs of chemosensitive and chemoresistant sublines with the same cell background, for response to erlotinib. Some of the chemoresistant variants showed greater sensitivity to erlotinib than the respective parental tumor cell line. We conducted several studies to try and understand the underlying mechanisms leading to enhanced erlotinib sensitivity in these chemoresistant sublines, such as that observed with CDDP-resistant ME180. Higher HER1/EGFR expression was found in seven more chemoresistant variant cell lines than parental cell lines; however, three of these showed enhanced sensitivity to erlotinib. HER1/EGFR expression may be required, but not sufficient for increased sensitivity to HER1/EGFR inhibitors. Moasser and colleagues (25, 28) observed an association between gefitinib inhibition of tumor growth and inhibition of PI3k/Akt activity in some tumor cell lines, particularly in HER2-overexpressing tumors. In our studies, erlotinib inhibited Akt activity in both CDDP-sensitive/erlotinib-resistant, and CDDP-resistant/erlotinib-sensitive, ME180 cell lines. Inhibition of Akt activity may not be the only determinant of sensitivity to growth inhibition by HER1/EGFR TKIs. We also showed that acquired resistance to different cytotoxic agents does not result in cross-resistance to erlotinib in the chemoresistant cell lines.

The observation that tumor cell lines with acquired resistance to chemotherapeutic drugs can develop increased expression of HER1/EGFR and increased sensitivity to HER1/EGFR TKIs may have clinical relevance. HER1/EGFR TKIs may be more effective in patients with certain types of chemoresistant tumors than patients with the corresponding chemo-naive tumors. Therefore, HER1/EGFR TKIs may be more effective as second- and third-line treatments for chemoresistant tumors. Alternatively, cancer patients with chemoresistant tumors may require higher concentrations of HER1/EGFR TKIs or a dosing regimen that involves an initial sequence of chemotherapy followed by HER1/EGFR TKIs either as monotherapy or in combination with chemotherapeutic agents. The complexity of HER1/EGFR activities on tumorigenicity mandates that further studies into the interactions between HER1/EGFR TKIs and chemotherapeutic agents are needed to improve the use of HER1/EGFR TKIs in the clinic.

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We thank Drs. Susan Horwitz, Li-Ying Yang, and Morhan Achary for providing us with some of the cell lines used in our experiments.

REFERENCES
Enhanced Sensitivity to the HER1/Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Erlotinib Hydrochloride in Chemotherapy-Resistant Tumor Cell Lines

Qun Dai, Yi-He Ling, Marie Lia, et al.


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