HER2 Overexpression Increases Sensitivity to Gefitinib, an Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor, through Inhibition of HER2/HER3 Heterodimer Formation in Lung Cancer Cells

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Abstract

Gefitinib (Iressa), an epidermal growth factor receptor targeting drug, has been clinically useful for the treatment of patients with non–small cell lung cancer (NSCLC). Gefitinib is currently being applied in clinical studies as either a monotherapy, or as part of a combination therapy against prostate, head and neck, gastric, breast, and colorectal tumors. However, success rates vary between different tumor types, and thus it is important to understand which molecular target(s) are responsible for limiting the therapeutic efficacy of the drug. In this study, we ask whether expression of HER2 affects sensitivity to gefitinib in human lung cancer cells. We established two clones, LK2/HER2-32 and LK2/HER2-57, by transfecting HER2 cDNA into LK2, a NSCLC line with a low expression level of HER2. We observed no mutations in exons 18, 19, and 21 of EGFR gene in LK2, LK2/mock- and two HER2-transfectants when we observed in-frame deletion mutations (E746-A750) adjacent to K745 in a gefitinib-sensitive NSCLC cell line, PC9. These LK2/HER2-32 and LK2/HER2-57 were more much more sensitive to the cytotoxic effects of gefitinib than the parental LK2 lines. Treatment with 0.5 to 1 μmol/L gefitinib specifically blocked Akt activation in both HER2-transfectant lines, but not in the parental LK2 cells. Extracellular signal-regulated kinase-1/2 activation, however, was not blocked by gefitinib up to 10 μmol/L in either the parent or transfectant lines. Gefitinib was also shown to induce cell cycle arrest in the G1-S phase, and an accompanying increase of p27Kip1 was observed. LK2/HER2 transfectants showed constitutive formation of HER2/HER3 heterodimer, which were seen to associate with a regulatory subunit of phosphoinositide-3-kinase, p85α, when active. Treatment of LK2/HER2 cells with gefitinib markedly decreased the formation of HER2/HER3 heterodimers, HER3 basal phosphorylation, and the association of p85α with HER3. This study is the first to show that under basal growth conditions, HER2 sensitizes low-EGFR NSCLC cell lines to growth inhibition by gefitinib. (Cancer Res 2005; 65(10): 4253-60)

Note: "Iressa" is a trademark of the AstraZeneca group of companies.

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very low dose of gefitinib of the drug-sensitive NSCLC cell line with EGFR mutations resulted in almost complete inhibition of EGFR autophosphorylation and the phosphorylation of its downstream targets, ERK1/2 and Akt (22).

On the other hand, overexpression of HER2 in various cancer cell lines or xenografts increases cytotoxicity and/or the antitumor effects of gefitinib (24–27). HER2/erbB2, a 185 kDa transmembrane protein tyrosine kinase, is overexpressed by gene amplification and constitutively activates cancer cell proliferation (28). Amplification of the HER2 gene has been reported in several types of cancer including breast, ovarian, stomach (29, 30), and NSCLC (31, 32), and most of these cases have been associated with poor prognosis (33–35). Of the HER family proteins, gefitinib inhibits EGFR/HER1 phosphorylation with an IC_{50} of 27 to 33 nmol/L, and HER2 phosphorylation with an IC_{50} of 3.7 μmol/L, suggesting about 100-fold difference in the drug sensitivity for EGFR and HER2 (11). HER3 is deficient in the tyrosine kinase domain and therefore shows no affinity to gefitinib. Because no studies seem to have addressed how HER2 overexpression modulates sensitivity to gefitinib in human cancer cells, we established isogenic cell lines with or without HER2 overexpression, in order to determine the mechanism of HER2 action. We have recently observed that one NSCLC cell line, LK2, expresses little, if any, EGFR and HER2, but moderately expresses HER3 (23). In our present study, we have established two sublines of LK2 (LK2/HER2) that have been transfected with wild-type HER2 cDNA, and we have examined how drug sensitivity to gefitinib is differentially controlled between LK2 and LK2/HER2.

Materials and Methods

Materials. The following materials were obtained from the indicated sources: Gefitinib (AstraZeneca, Macclesfield, United Kingdom); anti-HER2, anti-HER3, anti-p85α, and anti-phospho-HER2 antibodies (Upstate Biotechnology, Lake Placid, NY); antibodies to ERK1/2, phospho-HER1/2, Akt, phospho-Akt and p21^{WAF1/CIP1} (Cell Signaling Technology, Beverly, MA); anti-P-Tyr antibody (Santa Cruz Biotechnology, Santa Cruz, CA); anti-β-actin antibody, propidium iodide and RNase A (Sigma, St. Louis, MO); anti-p27^{kip1} antibody (BD Transduction Laboratories, San Jose, CA); anti-HER3 antibody for immunoprecipitation (NeoMarkers, Montreal, Quebec, Canada).

Cell culture. LK2 cells were purchased from Japanese Collection of Research Bioresources (Tokyo, Japan). PC9 and QG56 cells were kindly provided from Dr. Yukito Ichinose (Kyushu Cancer Center, Fukuoka, Japan). These cells were cultured in RPMI supplemented with 10% fetal bovine serum (23). LK2/HER2-32 and LK2/HER2-57 cells were established after stable transfection with pIREShyg2 expression plasmids (Clontech Laboratories, Palo Alto, CA) using Lipofectin 2000 Reagent (Invitrogen, San Diego, CA). These cells were cultured in RPMI supplemented with 10% fetal bovine serum and 350 μg/mL hygromycin, and were maintained under standard cell culture conditions at 37°C and 5% CO₂ in a humid environment.

Cell growth assay. Cell growth curves were determined by plating 5 × 10³ cells in a 24-well plate. After 24 hours, 20 ng/mL of EGF or vehicle was added, followed by incubation at 37°C. The medium was replaced every other day with fresh medium containing either EGF or vehicle, and cells were counted by a Coulter counter at indicated times.

Western blot and immunoprecipitation. Subconfluent tumor cells cultured in medium supplemented with 10% fetal bovine serum were incubated with various concentrations of gefitinib for 3 hours at 37°C. The cells were then rinsed with ice-cold PBS and lysed in Triton X-100 buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 1% Triton X-100, and 10% glycerol containing 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mmol/L sodium vanadate), and cell lysates were subjected to SDS-PAGE and transferred to Immobilon membranes (Millipore, Bedford, MA) as described previously (23, 36). After transfer, blots were incubated with the blocking solution and probed with anti-HER2 antibody, anti-HER3 antibody, anti-p85α antibody, anti-β-actin antibody, anti-phospho-HER2 antibody, anti-ERK1/2 antibody, anti-phospho-ERK1/2 antibody, anti-Akt antibody, anti-phospho-Akt antibody, anti-p21 antibody, or anti-p27 antibody followed by washing. The protein content was visualized using horseradish peroxidase-conjugated secondary antibodies followed by enhanced chemiluminescence (Amersham, Piscataway, NJ). For immunoprecipitation, 4 μg of total protein from cell lysates using NP40 buffer (50 mmol/L Tris-HCl, 1 mmol/L EDTA, 80 mmol/L NaCl, 0.3% NP40, and 10% glycerol containing 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mmol/L sodium vanadate) was incubated for 2 hours with anti-HER3 antibody and Protein A/G plus-agarose (Santa Cruz Biotechnology) and gently shaken. The precipitates were washed thrice with ice-cold lysis buffer and resolved by SDS-PAGE followed by Western blot analysis.

Cell viability assay. CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega, Madison, WI) was used to evaluate cytotoxicity in LK2 stable transfectants. One hundred microliters of an exponentially growing cell suspension (3–5 × 10³ cells) was seeded into a 96-well plate. The following day, various concentrations of gefitinib were added. After incubation for 72 hours at 37°C, 100 μL of CellTiter-Glo Reagent was added to each well and the plates were shaken gently for 2 minutes. After incubation for 10 minutes at room temperature, luminescence was measured using a multilabel counter (Wallac, Tokyo, Japan) (23, 36). Each experiment was done using three replicate wells for each drug concentration.

Colony formation assay. Cell survival was determined by plating 3 to 9 × 10³ cells in 35-mm dishes. After 24 hours, various concentrations of gefitinib were added, followed by incubation for 7 to 10 days at 37°C. Gefitinib was solubilized in DMSO. Control experiments were done by adding equivalent volumes of DMSO to plates. Colonies were counted after Giemsa staining as described previously (23).

Fluorescence-activated cell sorting analysis. The cells were seeded in a 6-cm dish and allowed to grow overnight. Gefitinib (1 or 5 μmol/L) was added for 48 hours at 37°C and cells were then and fixed overnight in ethanol at 4°C. Fixed cells were resuspended in a propidium iodide solution (15 μg/mL) containing RNase A (50 μg/mL) before incubation at room temperature for 1 hour. Cell cycle analysis was done using FACScan and Cell Quest software (Becton Dickinson Labware, Mountain View, CA).

Results

Overexpression of HER2 increases sensitivity to gefitinib in LK2 cells. First, we asked whether HER2 overexpression affects sensitivity to gefitinib by examining LK2/parent cells, LK2/mock cells, and HER2 cDNA transfectants. Two HER2 transfectants (LK2/HER2-32 and LK2/HER2-57) were isolated after introduction of human HER2 cDNA into LK2 cells expressing moderate levels of HER3, and very low levels of EGFR and HER2. These HER2 transfectants showed much higher HER2 expression than their parental counterparts, LK2 cells, and LK2/mock cells (Fig. 1A). We observed no apparent differences in the growth rates between LK2/mock and its two HER2 transfectants under exponential growth conditions in the absence or presence of EGF (Fig. 1B).

The expression levels of HER3 were similar among the four cell lines. Both LK2/HER2-32 and LK2/HER2-57 cells showed an approximately 2-fold greater sensitivity to the cytotoxic effects of gefitinib than their parental counterparts, when IC₅₀ values were determined by cell survival assay (Fig. 2A). Both HER2 transfectants showed similar sensitivities to gefitinib when assayed by cell survival assay. A separate assay, which was assessed by colony formation, showed LK2/HER2-57 cells to be approximately 5-fold more sensitive to gefitinib than LK2/mock cells and QG56 cells (Fig. 2B). However, sensitivity was about 20-fold lower in LK2/HER2-57 cells than in PC9 cells, which harbor in-frame deletion mutation of EGFR (E746-A750) in exon 19 (23). Our previous
research has shown that among nine NSCLC cell lines tested, PC9 is the most sensitive to gefitinib, and QG56 and LK2 are the most resistant (23). Thus, overexpression of HER2 seems to alter the sensitivity of LK2 cells to gefitinib.

The presence or absence of EGFR mutations plays a key role in the drug sensitivity of NSCLC cells to gefitinib (21, 22). We examined the absence or presence of any mutation in exons 18, 19, and 21 of EGFR gene, and found no mutation in LK2, LK2/mock, two HER2 transfectants and QG56 (data not shown).

Gefitinib inhibits Akt phosphorylation in a dose-dependent manner in LK2/HER2 cells. HER2 activates a number of cytoplasmic signal transduction pathways including the PI3K/Akt pathway and the Ras/MAP kinase pathway. We compared the effects of gefitinib on phosphorylation of HER2, Akt, and ERK1/2 in four cell lines: LK2/parent, LK2/mock, LK2/HER2-32, and LK2/HER-57. Figure 3A shows the effects of gefitinib on phosphorylation of HER2, Akt, and ERK1/2 in these cell lines under basal growth conditions in the presence of 10% serum. In all four cell lines, HER2, Akt, and ERK1/2 were phosphorylated in the absence of the drug (Fig. 3A). Moreover, P-Akt/2 in both LK2/HER2-32 and LK2/HER2-57 was about 1.3-fold higher than that in LK2/mock in the absence of drug (Fig. 3A). Furthermore, P-ERK1/2 in both LK2/HER2-32 and LK2/HER2-57 was about 1.3-fold higher than that in LK2/mock in the absence of drug (Fig. 3A).

Figure 3B shows inhibition dose kinetics of gefitinib for Akt and ERK1/2 in these cells, with the phosphorylation activity of these targets without gefitinib normalized as 100%. In LK2/parent cells and LK2/mock cells, activation of both Akt and ERK1/2 was not changed by gefitinib up to 10 μmol/L (Fig. 3A and B). However, in the two LK2/HER2 cell lines, Akt activation was inhibited by gefitinib in a dose-dependent manner, being blocked at 50% control on application of 1 μmol/L gefitinib. Application of 5 to 10 μmol/L gefitinib reduced HER2 phosphorylation by 40% to 60% of the activity in the absence of drug, whereas gefitinib did not show any inhibitory effect on ERK1/2 activation (Fig. 3A and B). Overexpression of HER2 thus enhanced drug sensitivity to gefitinib in LK2 cells and also specifically sensitized the gefitinib-induced inhibition of PI3K/Akt pathway.

Gefitinib arrests the cell cycle at G1 and increases p27Kip1 expression levels in LK2/HER2 cells. To examine whether the inhibitory effects observed in cell growth assays reflect a delay or arrest of cell cycle in the G0/G1 phase, as shown previously (37–39), cells were treated with gefitinib for 48 hours, and cell cycle progression was evaluated by fluorescence-activated cell sorting.
analysis. Treatment with gefitinib increased the portion of cells in G0/G1 phase by 7% to 8% in the two LK2/HER2 cell lines, with a corresponding decrease in the portion of cells in S and G2-M phases (Fig. 4A and B). In contrast, in LK2/mock cells, no change in cell cycle distribution was detected upon treatment with gefitinib. Furthermore, no sub-G1 fraction, indicative of apoptosis, was observed following gefitinib treatment in any of the cell lines. We subsequently examined the effects of gefitinib on p27^Kip1 and p21^{WAF1/CIP1} expression, because both of them have been implicated in the growth arrest after disruption of EGFR tyrosine kinase activity in EGFR or HER2 overexpressing cells (refs. 40, 41; Fig. 4C). In all cell lines, only a small amount of p27^Kip1 was expressed in the absence of the drug. Treatment with gefitinib (5 μmol/L) led to an increase in p27^Kip1 levels: approximately 8-fold in LK2/HER2-32 cells, and 4- to 5-fold in LK2/HER2-57 cells, when there was only a slight if any change in the p27^Kip1 levels in LK2/mock cells. In contrast, p21^{WAF1/CIP1} expression was unchanged in all of the clones after gefitinib treatment. Although inhibition by gefitinib in both growth curves and Akt activation was similar between LK2/HER2-32 and LK2/HER2-57, a slight but reproducible difference in an increase of p27^Kip1 was observed between the two gefitinib-treated transfectants. However, the underlying mechanism of why this difference in p27^Kip1 expression levels by gefitinib appears remains to be further studied.

**Gefitinib inhibits constitutive association of HER3 with HER2 as well as p85α, and basal HER3 phosphorylation in LK2/HER2 cells.** LK2 cells expressed no detectable levels of EGFR, but sufficient levels of HER2 (23). HER3 efficiently recruits p85α, but HER2 lacks the appropriate binding site(s) for this (42, 43). Moreover, the constitutive association of HER2/HER3 is often observed in tumor cells overexpressing HER2 (44). We examined the effect of gefitinib on the association of HER2 with HER3, and that of HER3 with p85α. LK2/HER2 and LK2/mock cells were treated with gefitinib for 3 hours and then cell lysates were immunoprecipitated with anti-HER3 antibodies, followed by Western blot analysis (Fig. 5). HER3 was coprecipitated with HER2 in LK2/HER2-32 and LK2/HER2-57 cells under basal growth conditions, indicating the presence of constitutive HER2/HER3 complexes. In LK2/mock cells, no HER2/HER3 heterodimer formation was apparent and HER3 exhibited only slight, if any, tyrosine phosphorylation and almost no

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**Figure 3.** Gefitinib inhibits Akt phosphorylation in a dose-dependent manner in LK2/HER2. A, exponentially growing cells in 10% serum medium were pretreated for 3 hours with the indicated concentrations of gefitinib. Protein extracts were resolved by 7.5% SDS-PAGE and probed with antibodies. HER2, Akt, and ERK1/2 activity was determined using each corresponding anti-phospho antibody. Relative levels of P-Akt and P-ERK1/2 in LK2/HER2-32 and LK2/HER2-57 are presented when basal phosphoprotein levels of each Akt and ERK1/2 in LK2/mock is presented as 100%. B, quantitative analysis of Akt and ERK1/2 activity was done on the immunoblots using Image Gauge V3.45 software and plotted for each cell line using the 0 μmol/L arm as a control.
In contrast, in LK2/HER2 cell lines, HER3 exhibited a high level of basal tyrosine phosphorylation and association with p85α. Treatment with gefitinib markedly abrogated HER3 phosphorylation as well as the association of HER3 with HER2, and HER3 with p85α.

**Discussion**

In a recent study, we examined the molecular basis of sensitivity to gefitinib using nine NSCLC cell lines, and we found that PC9 was most sensitive to the drug. These nine cell lines expressed different levels of EGFR, HER2, HER3, and HER4, but there appeared to be no correlation between EGFR and/or HER2 expression and drug sensitivity (23). However, the activation of EGFR, Akt and ERK1/2 was inhibited by much lower concentrations of gefitinib in PC9 cells than in the other eight cell lines under basal growth conditions. This suggests that sensitivity to growth inhibition by gefitinib in NSCLC cell lines is dependent upon the activation levels of Akt and ERK1/2 in response to EGFR signaling for survival and...

**Figure 4.** Gefitinib increases the proportion of cells in the G0/G1 phase of the cell cycle and increases p27Kip1 expression levels in LK2/HER2. A, cell cycle profiles of LK2/HER2 and LK2/mock at 48 hours after treatment with or without gefitinib are presented. Cell cycle distribution was determined by curve-fitting using the ModFit LT software. B, the result of cell cycle analysis of LK2/HER2 and LK2/mock. The mean values (SD) for each phase of the cell cycle are shown on the graph and in the table. C, exponentially growing cells were pretreated for 48 hours with the indicated concentrations of gefitinib. Protein extracts were resolved by 7.5% SDS-PAGE and probed with either antibody.
proliferation (23). Moreover, two established EGFR cDNA transfectants (LK2/EGFR-2 and LK2/EGFR-5) were found to show a similar level of sensitivity to gefitinib in the parental strain (23). In this study, overexpression of HER2 in LK2 cells is shown to result in enhanced drug sensitivity to gefitinib (Fig. 2A and B). Thus, the expression of HER2, but not EGFR, seems to modulate drug sensitivity to gefitinib in this NSCLC cell line. Therapeutic efficacy of gefitinib against NSCLC was found to be closely associated with somatic mutations in the EGFR kinase domain (21, 22), and we found that one highly gefitinib-sensitive NSCLC cell line, PC9, also has a deletion mutation (E746-A750) in exon 19 of EGFR catalytic domain. LK2, LK2/mock, and its two HER2 transfectants, had no such mutations in exon 18, 19, and 21 of the EGFR catalytic domain, suggesting that HER2 overexpression did not induce EGFR mutation in LK2 cells.

In HER2-transfected LK2 cells under basal growth conditions, application of 0.5 to 1 μmol/L gefitinib specifically inhibited Akt activation, but not ERK1/2 activation. Treatment with gefitinib induced G0/G1 arrest as well as the accumulation of p27Kip1 but not of p21WAF1/CIP1. The expression of CDK inhibitors, p27Kip1 and p21WAF1/CIP1, inhibits formation of the cyclin-CDK complexes essential for G1 to S phase progression (45). Inhibition of the EGFR-mediated pathway often induces up-regulation of both p21WAF1/CIP1 and p21WAF1/CIP1 (40, 41). In our present study, treatment with gefitinib of two HER2 transfectants resulted in no increase in p21WAF1/CIP1 expression but in up-regulation of p27Kip1 consistent with recent study by Kalish et al. (46). They have shown the down-regulation of cyclin D1 expression by gefitinib with concomitant accumulation of p27Kip1, but no change in the expression level of p21WAF1/CIP1. Accumulation of p27Kip1 protein rather than p27Kip1 protein seems to be specifically associated with growth arrest by gefitinib in LK2/HER2 transfectants.

Constitutive formation of HER2/HER3 heterodimers, and association of p85α with HER3 were observed, and gefitinib at 1 to 5 μmol/L markedly abrogated these associations. Taken together, overexpression of HER2 seems to sensitize LK2 cells to gefitinib, plausibly coordinated with HER3. A relevant study by Campiglio et al. (47) examined the effect of gefitinib on proliferation and survival, and its activation of Akt and ERK1/2, in six human breast cancer cell lines expressing various levels of EGFR and HER2. This study reported that the effects of gefitinib are independent of EGFR expression levels, but are influenced by high HER2 expression (47). Moreover, recent studies have also reported that gefitinib has a good antitumor effect on tumors displaying higher HER2 levels (24–26). These studies, including our present study, consistently suggest that high HER2 expression confers increased sensitivity to the therapeutic effect of gefitinib. Gefitinib intrinsically shows very high affinity against EGFR, but about 100-fold less affinity against HER2 than EGFR (11). IC50 of gefitinib for EGFR phosphorylation was about 0.1 to 0.5 μmol/L in various NSCLC lines (23), whereas IC50 of gefitinib for HER2 phosphorylation in LK2/HER2-32 and LK2/HER2-57 were about 10 μmol/L (Fig. 3). In contrast, gefitinib inhibited HER3 phosphorylation in the heterodimer HER2/HER3 at IC50 of about 1 μmol/L (Fig. 5), suggesting that HER3 is activated in the heterodimer with HER2, resulting in increased drug sensitivity to the inhibitory effect of gefitinib.

Overexpression of HER2 in LK2 cells resulted in a marked inhibitory effect of gefitinib on Akt phosphorylation, but not on ERK1/2 phosphorylation. HER2 phosphorylation under exponential growth conditions was moderately affected when gefitinib was used at high concentrations. Ras/ERK1/2 signaling is associated with cell proliferation, and Akt signaling is associated with cell survival (3, 48). Thus, HER2 seems to operate through Akt signaling, coordinated with HER3 and p85α in LK2/HER2 cells (see Fig. 6), and this signaling might be specifically affected by gefitinib. On the other hand, EGFR tyrosine kinase inhibitors often disrupt the formation of HER2/HER3 heterodimers in HER2-overexpressing cancer cells (25, 27), and we have shown that association of HER2 with HER3 in LK2/HER2 cells is markedly abrogated by gefitinib (Fig. 5). Expression of HER2 is essential for the dimer formation of HER2 and HER3, whereas exogenous addition of heregrin, a specific ligand for HER3, could not further enhance dimer formation or HER3 phosphorylation, in LK2/HER2 transfectants (data not shown). Treatment with gefitinib inhibited HER3 phosphorylation with release of p85α in LK2/HER2 cells under basal growth conditions. Concomitant disruption of HER2/HER3 formation by gefitinib, and the associated release of p85α, seems to specifically affect Akt signaling, resulting in growth arrest of cancer cells (Fig. 6).

Figure 5. Gefitinib inhibits the formation of HER2/HER3 heterodimers and the association of HER3 with p85α in LK2/HER2 cells. Exponentially growing LK2/HER2 or LK2/mock in 10% serum medium were treated with gefitinib (0.1 or 5 μmol/L) for 3 hours. After cell lysis, HER3 was immunoprecipitated with specific antibody. The immunoprecipitates were divided equally and subjected to immunoblot analysis using the indicated antibodies against HER3, P-Tyr, HER2, and p85α, respectively.
In conclusion a NSCLC cell line, LK2, has no apparent expression of EGFR and HER2, but expresses HER3 moderately, suggesting that HER2 (this study) as well as EGFR (23) does not seem to act as survival factors in LK2 cells. Cell proliferation and apoptosis in LK2 cells are expected to be driven by other growth factor receptors that are not targets for gefitinib (Fig. 6A). Overexpression of EGFR in LK2 cells resulted in no altered drug sensitivity to gefitinib (23). In contrast, overexpression of HER2 in LK2 cells results in enhanced drug sensitivity to gefitinib, and stimulates HER2-driven signaling accompanied by activation of Akt, plausibly through HER2/HER3 heterodimer formation (Fig. 6B). Cell survival and death, which are dependent on HER2/HER3 signaling, are then expected to be highly responsive to gefitinib treatment. Although our present study was done with artificially gene-modified cancer cells through wild-type HER2 cDNA transfection, Moasser et al. (24) have previously reported that gefitinib selectively inhibits HER2-driven signaling and suppress the growth of HER2-overexpressing breast and ovary cancer cell lines; these cancer cell lines were established from cancer patients without gene transfection. Further assessment of the activation levels of HER2 and/or HER3 could be useful in determining the therapeutic efficacy of the drug in a subgroup of NSCLC patients.

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**References**


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