Inhibition of HER2/neu (erbB-2) and Mitogen-activated Protein Kinases Enhances Tamoxifen Action against HER2-overexpressing, Tamoxifen-resistant Breast Cancer Cells

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ABSTRACT

HER2/neu (erbB-2) overexpression has been causally associated with tamoxifen resistance in human breast cancer cells. Forced expression of HER2 in MCF-7 breast cancer cells resulted in mitogen-activated protein kinase (MAPK) hyperactivity and tamoxifen resistance. Inhibition of HER2 and MAPKs with AG1478 and U0126, respectively, as well as dominant-negative MEK-1/2 constructs restored the inhibitory effect of tamoxifen on estrogen receptor (ER)-mediated transcription and cell proliferation. Both AG1478 and U0126 also restored the tamoxifen-mediated association of ER with nuclear receptor corepressor (N-CoR) in the antiestrogen-resistant MCF-7 cells. Treatment with a combination of tamoxifen and a HER2 kinase inhibitor reduced tumor MAPK activity and markedly prevented growth of HER2-overexpressing MCF-7 xenografts in athymic mice. Thus, blockade of HER2 and MAPK signaling may enhance tamoxifen action and abrogate antiestrogen resistance in human breast cancer.

INTRODUCTION

Estrogen-dependent breast cancer usually progresses from an antiestrogen-sensitive to an antiestrogen-resistant state. Several molecular and cellular mechanisms have been proposed as contributors to the acquisition of this resistant phenotype including the (infrequent) loss of ER,3 the selection of ER mutants, alterations in the intracellular pharmacology and/or binding of the antiestrogen tamoxifen to breast tumor cells, ligand-independent ER-mediated transcription, and perturbation of the interaction of ER with corepressors of transcription, among others (reviewed in Refs. 1–3). We have studied the role of HER2/neu (erbB-2) signaling in antiestrogen resistance. The HER2 receptor is the protein product of the HER2 proto-oncogene and a member of the EGFR (HER1) family of transmembrane tyrosine kinases, which also includes HER3 and HER4. Upon binding of ligands to the EGFR, HER3, or HER4, HER2 is recruited as the preferred partner of these ligand-bound receptors into active, phosphorylated heterodimeric complexes that activate several signaling pathways involved in the proliferation and survival of tumor cells.

Received 5/22/00; accepted 8/23/00.

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1 Supported by NIH Grant R01 CA80195, Department of Defense Breast Cancer Program Grant DAMD17-98-1-8262, a Clinical Investigator Award from the Department of Veteran Affairs (all to C. L. A.), and Vanderbilt-Ingram Cancer Center Support Grant CA68485. A. E. G. L. is the recipient of a Susan G. Komen Breast Cancer Foundation Program Grant DAMD17-98-1-8262, a Clinical Investigator Award from the Department of Defense Breast Cancer Program Grant DAMD17-98-1-8262, and a Clinical Investigator Award from the Department of Veteran Affairs (all to C. L. A.), and Vanderbilt-Ingram Cancer Center Support Grant CA68485. A. E. G. L. is the recipient of a Susan G. Komen Breast Cancer Foundation Fellowship Award.

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3 The abbreviations used are: ER, estrogen receptor; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; MEK, MAP kinase kinase; ERE, estrogen response element; N-CoR, nuclear receptor corepressor; P-Tyr, phosphorylated tyrosine; BrdUrd, 5-bromo-2'-deoxyuridine; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; PMSF, phenylmethylsulfonyl fluoride; FAAH, 6-carboxy-fluorescein; TAMRA, 6-carboxy-tetramethylrhodamine.
oiline EGFR/HER2 kinase inhibitor AG1478 was provided by J. Cherrington (Sugen, Inc., Redwood City, CA).

Colony-forming Assay. Growth effects of the different inhibitors were tested in an anchorage-independent soft agarose colony-forming assay. Tumor cells were plated at a density of 3 × 10^4 cells/35-mm dish in IMEM, 10% FCS, 0.8% agarose, 10 mM HEPES in the absence or presence of 4-OH tamoxifen, U0126, and AG1478, either alone or in combination. Dishes were incubated in a humidified CO2 incubator at 37°C and colonies measuring >70 μm in diameter were counted after 7 days using an OMNICON 3800 Tumor Colony Analyzer (Biologicals, Gainesville, VA).

Plasmids and Luciferase ER Reporter Assays. The pGL3-MERE and pGL3-MON plasmids (24) were provided by D. El-Ashty (Georgetown University, Washington, DC). Each plasmid contains either a double consensus ERE (MERE) or the same sequence with the ERE palindromes scrambled (MNON) into the HindIII site of pGL3 (Promega Corp., Madison, WI). The dominant-negative MEK-1 (pREP4-K97A MEK1) and dominant-negative MEK-2 (pREP4-K101A MEK2) plasmids (25) were provided by J. T. Holt (Vanderbilt University, Nashville, TN). MCF-7/HER2-18 and MCF-7/neo cells in 24-well plates were transiently transfected with 0.25 μg/well pGL3-MERE or pGL3-MON using 2.5 μl/well Lipofectamine reagent (Life Technologies, Inc.) in phenol red-free Opti-MEM I reduced serum medium (Life Technologies, Inc.) for 8 h. In some cases, 0.25 μg/well pREP4-K97A MEK1, pREP4-K101A MEK2, or pREP4 MEK1K2 plasmids (Invitrogen, Carlsbad, CA) were cotransfected with the ERE-containing plasmids. After an additional 24-h incubation in phenol red-free IMEM supplemented with 10% charcoal-stripped FCS (Cocalico Biologicals, Reamstown, PA), each well was replenished with fresh medium containing 1 μM 17β-estradiol with or without 1 μM 4-OH tamoxifen in the presence or absence of kinase inhibitors for 16 h. Firefly luciferase and Renilla reniformis luciferase activities in cell lysates were determined using the dual-luciferase reporter assay system (Promega) according to the manufacturer’s instructions in a Monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, CA). Firefly luciferase activity was normalized to Renilla reniformis luciferase activity and expressed as relative luciferase units.

Immunoprecipitation and Immunoblot Analysis. After washes with ice-cold PBS, cells were lysed in EBC buffer (50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.5% NP40, 100 mM NaF, 200 μM Na2VO4, 1 mM PMSF, 1 μg/ml pepstatin A, 2 μg/ml aprotinin, and 0.5 μg/ml leupeptin) for 20 min at 4°C. For analysis of ER and N-CoR, cells were lysed in a buffer containing 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 1% NP40, 0.2% Sarcosyl, 0.4 μg/ml NaCl, 20 mM NaF, 100 μM Na2VO4, 10 mM sodium molybdate, 1 mM PMSF, 1 μg/ml pepstatin A, 2 μg/ml aprotinin, and 0.5 μg/ml leupeptin as described previously (26). Cell lysates were centrifuged (14,000 rpm for 10 min at 4°C), and the protein content in the supernatants was determined by the BCA method (Pierce, Rockford, IL). Equal amounts of total protein were resolved by SDS-PAGE and then subjected to immunoblot analysis using polyclonal antibodies against erbB-2 (NeoMarkers, Freemont, CA), phospho-MAPK (Promega), total MAPK (New England BioLabs, Beverly MA), or N-CoR (Santa Cruz Biotechnology, Santa Cruz, CA), and an ERα monoclonal antibody (Novocastra laboratories, Burlingame, CA). In some cases, cell lysates were precipitated at 4°C with the erbB-2 antibody (10 μg/ml protein) and protein A-Sepharose CL-4B (Sigma). Immunocomplexes were then washed over to immunoblot analysis, as described, for HER2 or phosphotyrosine (P-Tyr; monoclonal antibody, Upstate Biotechnology, Lake Placid, NY). To detect an ER/N-CoR association, cell lysates were first precipitated with the N-CoR monoclonal antibody (GeneTex) and then precipitated with the ERα monoclonal antibody (Novocastra laboratories, Burlingame, CA). The sequences of the primer/probe sets used for each analysis are: ERG: F, 5′-TTCTCTGGTATCCCA-GAGGA-3′; R, 5′-AGCGTAATCCCAAGGATGT-3′; and P, 5′-FAM-AGAGCCAGCTCATCCTTTGGA-AA/MGTA-MRA-p-3′; HER2: F, 5′-TGTCAGCTGCGATGTTA-3′; R, 5′-TGCTCCCTGAGACACATCA-3′; and P, 5′-FAM-CAGAGGCGCAAGTCCGAGAAGCC-3′. HER2 analysis or to precipitation with HER2 antibodies, followed by P-Tyr immunoblotting. For histological analysis, portions of the excised tumors were fixed overnight in Histoprep (Fisher Scientific, Pittsburgh, PA), sectioned, and stained with H&E. BrdUrd labeling of tumor cell nuclei was visualized by staining the sections with BrdUrd antibody (Biogenics, San Ramon, CA), as indicated by the Histo-mouse kit (Zymed Laboratories, Inc., South San Francisco, CA). Additional sections were used to stain for apoptotic cells using the DNA in situ nick end-labeling (TUNEL) method (Intergen).

RESULTS

HER2 Overexpression Inhibits Tamoxifen Action against ER-positive MCF-7 Cells. We have examined the mechanisms by which overexpression of HER2 confers antiestrogen resistance to breast tumor cells. We used MCF-7 cells stably transfected with a full-length
HER2 cDNA (MCF-7/HER2–18), which exhibit 45-fold higher levels of HER2 protein and acquire tamoxifen resistance relative to control cells (6). Confirming previous reports, the antiestrogen 4-OH tamoxifen inhibited anchorage-independent growth of MCF-7 control cells transfected with vector alone (MCF-7/neo) in a dose-dependent manner. In a colony-forming assay, control cells exhibited 80–83% inhibition of colony formation, whereas MCF-7 cells with high HER2 levels were modestly inhibited by 0.1–1 μM 4-OH tamoxifen (Fig. 1A). By immunoblot analysis, ER levels in MCF-7/HER2–18 cells and in the tamoxifen-sensitive MCF-7/neo and parental MCF-7 cell lines were similar (Fig. 1B). To rule whether transfection of HER2 had modified the levels of homologous EGF receptor-type kinases, we used a quantitative PCR (TaqMan; Refs. 29 and 30). Relative to a housekeeping ribosomal protein L19 (RPL19) gene, only the HER2 mRNA (13-fold), but not EGFR (HER1), HER3, or HER4, was found to be in excess in MCF-7/HER2–18 cells (Fig. 1C). Antiestrogen resistance was also documented by examining transcriptional responses of luciferase reporter constructs containing ERE. Addition of 1 nM estradiol activated ERE-luciferase transcription 18.7-fold in MCF-7/HER2–18 and 5.3-fold in MCF-7/neo cells above each control (Fig. 2). Because of different transfection efficiencies, a comparison of the constitutive ER-mediated transcription between both lines with high and low HER2 levels cannot be done. The difference in the level of estrogen-induced transcription did not translate into a difference in estrogen-stimulated cell proliferation in that both cell lines exhibited a similar 2–3-fold increase in cell number in response to estradiol in estrogen-free medium after 4 days (data not shown). Addition of 1 μM 4-OH tamoxifen suppressed estradiol-induced ERE-luciferase transcription 26% (P = 0.28) and 58% (P < 0.001) in MCF-7/HER2–18 and MCF-7/neo cells, respectively (Fig. 3). Of note, tamoxifen alone increased ERE-luciferase transcription 1.5-fold above basal in MCF-7/HER2–18 but not in control cells. These data indicate that both HER2-overexpressing and control MCF-7 cells exhibit wild-type ER function in response to exogenous ligand but a clear difference in sensitivity to tamoxifen in both growth and transcription assays.

**Blockade of HER2 and MAPK Restores Transcriptional and Cellular Responses to Tamoxifen in Vitro.** We next examined whether blocking HER2 signaling with pharmacological and genetic approaches would abrogate antiestrogen resistance. For this purpose, we used: (a) AG1478, a small molecule quinazoline that inhibits the EGFR and HER2 kinases with an in vitro IC50 of 3 nM and 1.4 μM, respectively (27); and (b) U0126, a noncompetitive inhibitor of the dual specificity MAP kinase kinases (MEK-1/MEK-2), the enzymes that activate MAPK (28). By immunoblot analysis, constitutively phosphorylated HER2 and active MAPK were only detectable in MCF-7/HER2–18 but not in MCF-7 and MCF-7/neo cells. 4-OH tamoxifen had no effect on phosphorylated HER2 or active MAPK. However, AG1478 eliminated both HER2 phosphorylation and active MAPK, whereas U0126 eliminated only active MAPK in MCF-7/HER2–18 without altering total HER2 or total MAPK protein levels (Fig. 3A). To explain the inhibitory effect of AG1478, a relatively specific EGFR kinase inhibitor, on HER2 phosphorylation, we examined whether AG1478 would interact with HER2 in MCF-7/HER2–18 cells. A brief 1-h incubation with 1–10 μM AG1478 induced the formation of HER2 dimers. At the higher dose of AG1478, these HER2 dimers lacked a P-Tyr signal (Fig. 3B), potentially explaining the inhibitory effect of AG1478 on HER2 phosphorylation shown in Fig. 3A. Finally, we examined the target specificity of the kinase inhibitors in an in vitro kinase assay using exogenous ERK-1 (p44/MAPK) as a substrate for MEK-1 immunoprecipitated from MCF-7/HER2–18 cells. U0126 but not AG1478 inhibited MEK-1 induced phosphorylation of ERK-1 in vitro (Fig. 3C), suggesting that the inhibitory effect of AG1478 on MAPK activity in intact cells (Fig. 3A) is not attributable to a nonspecific interaction of AG1478 with MEK-1.

We next determined whether inhibition of HER2 and MAPK would restore tamoxifen effects on ER-mediated transcription and cell

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growth. 4-OH tamoxifen, AG1478, or U0126 did not appreciably inhibit estradiol-stimulated ERE-luciferase reporter activity in MCF-7/HER2–18 cells. However, both AG1478 and U0126 significantly enhanced the inhibitory effect of tamoxifen on ER-mediated luciferase expression in a dose-dependent fashion (Fig. 4A). To support that this enhancement of tamoxifen action was attributable to the interruption of MAPK activity, we used constructs encoding dominant-negative MEK-1 and MEK-2 (25). Relative to control vector, transiently transfected dominant-negative MEK-2 or the combination of dominant-negative MEK-2 and MEK-1 mutants significantly enhanced tamoxifen-mediated inhibition of ER-induced luciferase expression (P < 0.05; Fig. 4B), whereas the effect of dominant-negative MEK-1 approached statistical significance (P = 0.1; Fig. 4B). It has been proposed that tamoxifen blocks estrogen action by recruiting transcriptional corepressors to the ligand-binding domain in the ER (32). Hence, we examined the association of ER with the N-CoR in both MCF-7/neo and MCF-7/HER2–18 cells. ER and N-CoR content in these cells was similar. Tamoxifen induced a strong association of ER with N-CoR in the antiestrogen-sensitive MCF-7/neo cells, whereas this effect was markedly reduced in MCF-7/HER2–18 cells (Fig. 5). However, treatment of the tamoxifen-resistant cells with AG1478 or U0126 enhanced tamoxifen-mediated association of N-CoR with ER without any alterations in ER and N-CoR levels. These results are consistent with the transcriptional responses shown in Fig. 4.

Finally, we tested whether the transcription data correlated with tumor cell growth. 4-OH tamoxifen (0.1 μM) inhibited MCF-7/HER2–18 colony formation 40% compared with untreated controls. Addition of AG1478 or U0126 increased tamoxifen-mediated growth inhibition to similar levels (80% below control) to those seen in MCF-7/neo cells (Fig. 6A). Similar data were obtained with ER-positive BT-474 cells that exhibit gene amplification and constitutive phosphorylation of the HER2 receptor (33) with an IC50 to tamoxifen positive BT-474 cells that exhibit gene amplification and constitutive (33). Similar data were obtained with ER-positive BT-474 cells that exhibit gene amplification and constitutive phosphorylation of the HER2 receptor (33) with an IC50 to tamoxifen positive BT-474 cells that exhibit gene amplification and constitutive phosphorylation of the HER2 receptor (33).

Inhibition of HER2 Signaling in Vivo Restores Tamoxifen Sensitivity. The effect of HER2 blockade with AG1478 on tamoxifen action was next tested against MCF-7/HER2–18 xenografts in athymic mice. Although estrogen responsive, these cells form tumors in the absence of added estradiol (6). To accelerate tumor establishment, mice were supplemented with 17β-estradiol delivered as a s.c. pellet. Once xenografts had reached a volume ≥200 mm3, tumor-bearing mice were randomly allocated to no treatment or treatment with tamoxifen, AG-1478, or both. Tamoxifen minimally delayed tumor growth (P = 0.23), whereas AG1478 had no effect. However, the
combination of tamoxifen and AG1478 almost completely arrested tumor growth relative to untreated controls (P < 0.01; Fig. 7A). To determine whether the effect of this combination was antiproliferative versus cytotoxic, three mice/group were pulsed with BrdUrd prior to tumor harvesting. Compared with control tumors, there was a significant reduction in the number of BrdUrd-labeled nuclei in the tumors treated with tamoxifen plus AG1478 (P < 0.01) but not in the other two experimental groups (Fig. 7B). Histologically, tumors from all groups were identical. To evaluate whether programmed cell death was occurring as a result of treatment, tumor sections were evaluated for the presence of apoptotic cells in situ as measured by TUNEL assay. A modest increase in TUNEL-positive tumor cells was only observed in the sections from xenografts treated with the combination compared with control tumor cells (P = 0.06; Fig. 7B). Finally, to validate our molecular targets in vivo, we examined whether HER2 signaling had been inhibited in control and treated tumors. A representative cohort is shown in Fig. 7C. Levels of HER2 and total MAPK were similar in tumors from all four experimental groups. However, phosphorylated HER2 and active MAPK were markedly reduced in the tumors treated with AG1478, suggesting that HER2 signaling had been disabled by AG1478 in vivo. Overall, these data suggest that interference of the HER2 signaling pathway enhances tamoxifen action against antiestrogen-resistant cells both in vitro and in vivo.

**DISCUSSION**

We have studied the role of HER2 signaling in antiestrogen resistance in an MCF-7 subline that over expresses the HER2 proto-oncogene. The cells exhibit constitutively active MAPK and an ~100-fold higher IC50 to tamoxifen compared with MCF-7 cells with a single copy of the HER2 gene. In this model, ER content and function, as measured by estradiol-induced responses of ER reporters and cell proliferation, are maintained. These data suggest that the differences in tamoxifen response in this model are not attributable to differences in ER levels but to the potential interaction of signal transduction elements downstream from HER2 with the ER. To disengage HER2 function, we used the small molecule kinase inhibitor AG1478, which displays an IC50 against EGF-stimulated phosphorylation of an
HER2 dimers (Fig. 3B), suggesting that it may interact directly with the HER2 kinase. By inducing the formation of inactive, unphosphorylated HER2 dimers, it can sequester the receptor from functional interactions with other HER receptor partners and, therefore, disrupt signaling. Treatment of intact cells with AG1478 eliminated active MAPK (Fig. 3A) but had no effect on MEK-1 activity in vitro (Fig. 3C), suggesting that the effect of the quinazoline on MAPK occurred as a result of the interruption of HER2 signaling upstream.

Several issues suggested the possibility that the EGFR kinase may have been involved in the antiestrogen-resistant phenotype of MCF-7/HER2–18 cells: (a) AG1478 has a predominant effect against the EGFR kinase (IC_{50}, 3 nM; Ref. 27); and (b) transfection of EGFR into hormone-dependent breast cancer cells also induces tamoxifen resistance (34, 35). Moreover, HER2 can prolong EGFR stability and both receptors contribute to transformation in a cooperative manner (4, 5, 36). However, we were unable to detect EGFR by immunoblot analysis of EGFR precipitates from MCF-7/HER2–18 cell lysates. In addition, the C225 antibody against the EGFR ectodomain (37) had no effect on colony survival of the HER2-transfected MCF-7 cells (data not shown). Moreover, EGFR mRNA levels, as measured by real-time quantitative PCR, were low in HER2-transfected and control cells (Fig. 1C). Hence, although we cannot rule out some contribution of the EGFR to tamoxifen resistance in our experimental system, we do not feel that this kinase is playing a major causal role. There are no published reports implicating the homologous family members HER3 and/or HER4 with loss of estrogen dependence or antiestrogen resistance in breast cancer cells. Nonetheless, the levels of these mRNAs were not altered by HER2 transfection into MCF-7 cells (Fig. 1C).

The following results implicate MAPK signaling as the mediator of antiestrogen resistance in HER2-overexpressing tumor cells: (a) overexpression of HER2 in MCF-7 cells results in both activation of MAPK and antiestrogen resistance; (b) inhibition of MAPK with U0126 enhances the ability of tamoxifen to inhibit both ER-mediated transcription and cell growth; (c) dominant-negative mutants of MEK-1 and MEK-2 also enhance the inhibitory effect of tamoxifen on ER-mediated transcription; and (d) AG1478 markedly reduces active MAPK in MCF-7/HER2–18 xenografts, and this reduction is temporally associated with tamoxifen-induced growth restraint of tumors in vivo. Neither HER2 overexpression (in MCF-7/HER2–18 cells) nor inhibition of HER2 and MAPK altered ER levels or reporter activity, suggesting that the restoration of tamoxifen action was independent of ER levels and function. In a more recent report, however, another HER2-transfected MCF-7 cell line (8) exhibited significant down-regulation of ER protein. Inhibition of MEK or MAPK restored ER expression and, presumably, sensitivity to antiestrogens (38). This result suggests that HER2 hyperactivity may subvert the antiestrogen response by multiple potential mechanisms.

Notably, treatment with AG1478 alone inhibited tumor MAPK but not tumor growth (Fig. 7). This result raises the intriguing possibility that estrogen-primed tumor growth is not dependent on MAPK and antiestrogen resistance. This possibility will require further studies with MAPK inhibitors in combination with tamoxifen. In addition, tumors treated with AG1478 alone and in which P-MAPK was reduced (Fig. 7C) were harvested 2 h after treatment with AG1478. Thus, it is conceivable that the combination of tamoxifen plus AG1478 induced a more sustained down-regulation of MAPK function compared with treatment with the kinase inhibitor alone. This speculation requires further investigation beyond the scope of this report. Our in vitro studies on the role of MAPK would seem to be in some disagreement with a recent report by Lobenhofer et al. (39) in which estrogen-dependent cell proliferation and transcription were inhibited by 25 μM U0126 over a 24-h
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It should be noted, however, that these concentrations were ~10-fold higher than those of U0126 that inhibited ERK-1 activity (Fig. 3C) and P-MAPK content (Fig. 3A), which enhanced the inhibitory effect of tamoxifen on ER reporter activity (Fig. 4A) in our study. Therefore, these discrepancies could be explained by the different concentrations of U0126 that were used.

MAPK has been shown to posttranslationally modify the ER by phosphorylating Ser-118. The evidence that p42/44 MAPK directly phosphorylates Ser-118 is based on the in vitro observation that activated MAPK phosphorylates the AF-1 domain in the NH₂ terminus of the ER but not a mutant in which Ser-118 was replaced by Ala (21, 22, 40). In some cell types, this mutation causes a reduction in estradiol-dependent transcriptional activation (21, 41). The robust activation of ERE-luciferase expression in MCF-7/HER2–18 cells (Fig. 2) would argue against a mutation in AF-1 in the HER2-overexpressing cells. In addition, we have not been able to detect phosphorylated ER bands in MCF-7/HER2–18 or BT-474 cells maintained in estrogen-containing medium, despite the use of optimal conditions to inhibit cellular phosphatases (42), implying that under conditions in which MAPK is hyperactive (Fig. 3), Ser phosphorylation may not be abundant in HER2-overexpressing tumor cells.

Inhibition of HER2 and MAPK also sensitized BT-474 human breast cancer cells to tamoxifen (Fig. 6). These cells exhibit HER2 gene amplification, a constitutively active HER2 kinase, and at best a 50% growth inhibition in the presence of high (1 μM) concentrations of 4-OH tamoxifen. Preliminarily, we have also observed a modest synergetic inhibitory effect of tamoxifen and AG1478 in ER-positive MDA-361 human breast tumor cells, which exhibit HER2 overexpression (33) but no HER2 gene amplification in the MCF-7/HER2–18 cells, which exhibit HER2 overexpression. Preliminarily, we have also observed a modest synergetic inhibitory effect of tamoxifen and AG1478 in ER-positive MDA-361 human breast tumor cells, which exhibit HER2 overexpression. Preclinical studies in the in vivo model of breast cancer have demonstrated that inhibition of HER2 and MAPK signaling may also enhance tamoxifen action on hormone-dependent, tamoxifen-sensitive breast tumor cells that do not overexpress HER2, as suggested recently by Kunisue et al. (43) in studies using the pure antiestrogen ICI 182,780 and another humanized HER2 antibody. The exquisite sensitivity of wild-type and MCF-7/neo (control) cells to concentrations as low as 0.04 μM tamoxifen makes these cell lines unsuitable to address this important question.

Be that as it may, the relevance of our results to breast tumor cells with normal levels of HER2 or more modest levels of HER2 overexpression will require further research.

It has been proposed that tamoxifen recruits transcriptional corepressors to the AF-2 region in the hormone-binding domain of the ER to block ER-mediated transcription (3, 32). Mitogens such as protein kinase A, dopamine, or EGF can decrease this antiestrogen-induced receptor-corepressor interaction and limit tamoxifen action (44–46). Thus, we examined whether HER2 signaling, which activates the same signaling pathways activated by EGF (4, 5), can also disrupt this interaction. Notably, tamoxifen-induced association of ER with N-CoR was reduced in HER2-overexpressing cells. Inhibition of HER2 and MAPK markedly increased the antiestrogen-mediated association of ER with N-CoR. This result implies that antiestrogen resistance in HER2-overexpressing tumor cells can, at least in part, be attributed to unstrained MAPK signaling that alters the ER association with corepressors of transcription. The molecular mechanisms by which MAPK regulates these protein-protein associations require further study. Nonetheless, the data presented imply that: (a) MAPK may play a causal role in HER2-induced tamoxifen resistance in ER-positive breast tumor cells; and (b) that exogenous inhibitors of HER2 signal transduction can partially abrogate the antiestrogen-resistant phenotype, providing a testable therapeutic strategy in hormone-dependent human breast cancer.

ACKNOWLEDGMENTS

We thank Teresa C. Dogger, Mary Beth Carter, and Sandy Olson for outstanding technical assistance.

REFERENCES


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