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Her2 cross talk and therapeutic resistance in breast cancer

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TABLE OF CONTENTS

1. ABSTRACT

The HER2 receptor tyrosine kinase is amplified and/or overexpressed in approximately 30% of metastatic breast cancers. Interactions and cross signaling from the HER2 receptor to other growth factor receptors may potentially contribute to therapeutic resistance. In this review, we discuss HER2 receptor cross talk with the estrogen receptor and implications toward resistance to endocrine therapies. We also review mechanisms of resistance to the HER2-targeted antibody trastuzumab, including signaling from other members of the HER family, increased signaling through the PI3-kinase pathway, and cross talk from the insulin-like growth factor-I receptor to HER2. Finally, we will provide perspective on how HER2 receptor cross talk may provide critical information for developing novel therapeutic options for HER2-overexpressing breast cancers.

2. INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor (EGF) or erbB family of receptor tyrosine kinases, which also includes HER3 and HER4. HER3 is the only member that lacks intrinsic tyrosine kinase activity, and HER2 is unique in that it does not have a known ligand. When ligands bind to the EGF receptor (EGFR), HER3, or HER4, receptor dimerization occurs, with HER2 being the preferred dimerization partner (1). Dimerization activates multiple downstream signaling cascades including the mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways, which promote cellular proliferation, survival, migration, invasion, and differentiation.

Overexpression of HER2 occurs in approximately 30% of metastatic breast carcinomas, due

primarily to gene amplification, and is associated with an increased tendency for metastasis and reduced disease-free and overall survival (2, 3). HER2 is an attractive therapeutic target in breast cancers because of the tight correlation between overexpression and poor prognosis, and because normal cells express relatively low levels of HER2. Trastuzumab (Herceptin), a recombinant humanized monoclonal antibody directed against the extracellular domain of HER2 (4), was approved by the United States Food and Drug Administration (U. S. F. D. A.) in 1998 for clinical use against metastatic breast cancers that overexpress HER2. Response rates to trastuzumab monotherapy range from 12% to 34% with median duration of 9 months (4, 5). Combination with chemotherapeutic agents increases response rates, but duration remains under one year. Thus, resistance to trastuzumab is a significant clinical problem.

To improve available treatments for HER2-overexpressing breast cancer, a better understanding of the mechanisms contributing to response and resistance to trastuzumab is needed. In addition, the mechanisms of HER2 signaling and interactions with other pathways must be examined to more effectively treat HER2-overexpressing breast cancers. In this review, we will discuss recent studies that demonstrate interaction and cross talk between HER2 and other growth factor receptor pathways and therapeutic implications.

3. MECHANISMS OF HER2-DEPENDENT TUMORIGENESIS

Trans-phosphorylation by HER2 appears to be an important mechanism by which this receptor tyrosine kinase promotes tumorigenesis. This is evident within the erbB family itself, as HER2 is frequently co-expressed with HER3 and EGFR in breast cancer cells. The HER2/HER3 dimer is thought to be the most potent signaling complex in the erbB family, resulting in direct activation of the PI3K pathway. The transforming capacity of HER2 was increased synergistically in the presence of HER3 (6), while loss of HER3 prevented proliferation of HER2-overexpressing breast cancer cells (7). *In vivo*, HER3 expression was not only increased in transgenic HER2 mouse models, but was actually tyrosine phosphorylated, suggesting that HER2 phosphorylates HER3 (8). Importantly, cells that lack HER3 expression were insensitive to the murine precursor of trastuzumab, 4D5 (9). Thus, interaction and phosphorylation of HER3 appears to be essential for HER2-mediated mammary cell proliferation and tumorigenesis. In addition to interacting with and phosphorylating members of its receptor family, HER2 also activates other receptor signaling pathways.

4. ENDOCRINE RESISTANCE

Approximately two-thirds of primary breast cancers express the estrogen receptor alpha isoform (ER). Modulators of ER activity represent the first example of targeted anti-neoplastic therapy. Tamoxifen, a non-steroidal serum estrogen receptor modulator (SERM), was the first ER-targeted agent to be approved for clinical use. Tamoxifen is a competitive inhibitor of estrogen binding to the ER, and induces a receptor conformational change such that ER transcriptional activity is repressed. Other ER modulators used clinically for the treatment of ER-positive breast cancers include aromatase inhibitors and steroidal anti-estrogens such as ICI 182,780 (Faslodex) (10).

Those breast cancers that show low or absent ER expression display primary resistance to ER-targeted therapies. A subset of ER-positive breast cancers also demonstrates primary resistance, while the majority of tumors that initially respond to ER modulators eventually acquire resistance. Hence, endocrine resistance is a major clinical problem in the treatment of breast cancer. ER expression and function are generally intact in endocrine-resistant breast tumors, as supported by data showing full DNA binding capability of ER with subsequent transcriptional activity in resistant cells (11). Mutations in the ER are rare, although a point mutation resulting in a hypersensitive receptor capable of responding to low levels of estrogen stimulation was previously noted in a pre-malignant proliferative lesion of the breast (12). Mechanisms leading to the development of endocrine resistance are not completely understood, but current data strongly supports a role for growth factor receptor cross talk to the estrogen receptor.

4.1. HER2/estrogen receptor cross talk

Long-term exposure of MCF7 ER-positive breast cancer cells to tamoxifen produced resistant clones that expressed ER levels equivalent to tamoxifen-sensitive parental MCF7 cells (13). These tamoxifen-resistant cells demonstrated increased levels of phosphorylated and total EGFR and HER2, as well as of downstream ERK1/2 (13, 14). Similarly, long-term culture of MCF7 cells in the anti-estrogen ICI 182,780 yielded resistant cells with heightened EGFR signaling and increased MAPK activation (15). The EGFR-targeted tyrosine kinase inhibitor (TKI) gefitinib (ZD1839; Iressa) completely blocked basal and EGF/TGF alpha-stimulated ERK1/2 activation and inhibited growth of tamoxifen-resistant cells (13, 14). Suppression of HER2 activity has also been shown to increase tamoxifen-mediated inhibition of breast cancer cell proliferation (16). Furthermore, transfection of HER2 rendered ER-positive breast cancer cells estrogen-insensitive and tamoxifen-resistant, with the HER ligand heregulin (HRG) stimulating a direct and rapid phosphorylation of ER (17). EGF has also been shown to stimulate phosphorylation of ER in MCF7 HER2 stable transfectants (MCF7/HER2), with gefitinib blocking both HRG- and EGF-induced ER phosphorylation (18). Conversely, in these same cells, estrogen and tamoxifen both stimulated phosphorylation of EGFR and HER2, demonstrating that tamoxifen acts as an agonist in the setting of elevated HER2 expression; again, these effects were blocked by gefitinib (18). BT474 breast cancer cells, which possess endogenous overexpression of HER2 due to gene amplification, showed similar effects in that estrogen and tamoxifen induced HER2 phosphorylation, which was blocked by gefitinib (18). Interestingly, in contrast to MCF7 parental cells, MCF7/HER2 demonstrated interaction between HER2 and ER with cytoplasmic localization of ER and increased

phospho-ERK1/2 (16). Estrogen stimulation increased this receptor interaction, whereas ICI 182,780 disrupted the ER/HER2 complex and promoted nuclear localization of ER (16). Collectively, these data indicate that ER and HER2 have a bidirectional cross talk which leads to tamoxifen resistance or conversion of tamoxifen to an ER agonist.

In vivo data also supports a role for EGFR/HER2 signaling and ER cross talk in endocrine resistance. Xenografts of MCF7/HER2 were growth inhibited by estrogen deprivation and growth stimulated by tamoxifen, demonstrating interaction between the HER2 and ER pathways and resistance to tamoxifen in ER-positive HER2-overexpressing MCF7 cells (18). Gefitinib suppressed tamoxifen-induced growth of MCF7/HER2 xenografts (18). Comparison of MCF7/HER2 cells with parental MCF7 cells showed cross-phosphorylation of ER and EGFR/HER2 signaling in a bidirectional manner. These cells also showed increased phosphorylation of ERK1/2, Akt, and the ER co-activator AIB1/Src-3 (amplified in breast cancer 1; Src-3, steroid receptor co-activator-3). Importantly, patients with tamoxifen-treated ER-positive breast cancers showing high expression levels of both AIB1 and HER2 had the highest rate of recurrence versus tumors expressing neither or only one of AIB1 or HER2 at high levels (19). Similarly, elevated levels of AIB1, HER2, and HER3 or increased expression of one of EGFR, HER2, or HER3 in combination with high levels of AIB1 increased the risk of relapse on tamoxifen treatment. In this latter study (20), HER2 and AIB1 co-overexpression exhibited approximately 2-fold increased risk of relapse, while HER3 and AIB1 co-overexpression showed a 3.7-fold increased risk of relapse for tamoxifen-treated patients. Thus, EGFR family members promote resistance to endocrine agents in the presence of the ER coactivator AIB1.

Clinical trials have examined inhibition of growth factor signaling as a therapeutic strategy in endocrine-resistant breast cancer. Based on evidence suggesting that cross talk between the estrogen and HER2 pathways promotes endocrine therapy resistance, the aromatase inhibitor (AI) letrozole was combined with trastuzumab in a phase II trial of ER+/HER2+ advanced breast cancer patients (21). The overall response rate was 26% with a clinical benefit rate of 52%, suggesting possible benefit from combination trastuzumab and letrozole in patients with ER+ and HER2+ breast cancer. However, the molecular mechanisms mediating response versus resistance in this population need to be determined, as approximately half of the patients did not respond. Another, more recent phase II trial of gefitinib with neoadjuvant AI anastrozole showed no additional clinical benefit versus anastrozole alone in patients with early (stage I to IIIB) ER+ breast cancer (22). Hence, inhibition of HER family growth factor signaling with targeted therapies appears to be an attractive strategy, at least for a subset of endocrine-resistant metastatic or advanced breast cancers that are ER+ and HER2+, warranting further study in this population.

5. TRASTUZUMAB RESISTANCE

Approximately one-third of HER2-overexpressing metastatic breast cancers respond to single-agent trastuzumab (4,5), with almost two-thirds responding to combination taxane - trastuzumab regimens (23,24). However, responses are short-lived, averaging less than one year (4,5,23,24). In the adjuvant setting, administration of trastuzumab in combination with or following chemotherapy improves disease-free and overall survival rates in patients with early-stage breast cancer (25-27). However, approximately 15% of patients still develop metastatic disease despite trastuzumab-based adjuvant chemotherapy. Thus, both de novo and acquired trastuzumab resistance are significant clinical problems in metastatic and early disease. Preclinical studies examining the molecular mechanisms contributing to trastuzumab resistance are important in order to achieve a greater response rate, and to identify novel agents that will benefit trastuzumab-refractory breast cancer patients.

Most preclinical models have reported that her2 amplification and protein overexpression are maintained in trastuzumab-resistant derivatives of HER2-overexpressing cells (28,29), indicating that the molecular target (HER2) is still intact. Specific mechanisms resulting in trastuzumab resistance are not fully understood, but several proposed models now exist, including but not limited to the inability of trastuzumab to block HER heterodimerization or signaling from other HER receptors (29-32), increased PI3K signaling (33-35), insulin-like growth factor-I receptor (IGF-IR) signaling (28, 36-37), and truncated, kinase-active forms of HER2 (39). Interactions of HER2 signaling with other signaling pathways as a mechanism of trastuzumab resistance will be reviewed below.

5.1. HER family signaling

Although trastuzumab is able to diminish signaling downstream of HER2, it does not disrupt heterodimer formation between HER2 and other HER family receptors. Hence, compensatory signaling downstream of HER heterodimers occurs and can potentially contribute to trastuzumab resistance. EGFR, HER3, or HER4 ligands EGF, TGF (transforming growth factor)-alpha, betacellulin, and HRG have been shown to increase downstream HER signaling and prevent trastuzumab-mediated growth inhibition (29-32). Ritter *et al.* (29) recently reported that trastuzumab-resistant BT474-derived cells expressed elevated protein levels of EGFR/HER2 heterodimers and phosphorylated EGFR, and increased transcripts for the HER ligands EGF, TGF-alpha, heparin-binding EGF, and HRG. The EGFR-directed kinase inhibitors erlotinib and gefitinib and the dual EGFR/HER2 kinase inhibitor lapatinib suppressed growth of trastuzumab-resistant cells and inhibited phosphorylation of EGFR, HER2, and HER3. Lapatinib has also been shown by other groups (40,41) to be effective in trastuzumab-refractory breast cancer cells, suggesting possible translational therapeutic relevance to these findings. Data showing increased signaling by HER receptors and increased levels of HER ligands as a potential mechanism of resistance suggest that monitoring levels of HER ligands in HER2-overexpressing patients treated with trastuzumab may be a useful strategy for predicting response.

5.2. PI3K signaling

Constitutive PI3K signaling has been shown to inhibit cell cycle arrest and apoptosis mediated by trastuzumab (33). In addition, trastuzumab-resistant cells derived from the BT474 HER2-overexpressing breast cancer line demonstrated elevated levels of phosphorylated Akt and Akt kinase activity compared with parental cells (42). These resistant cells also showed increased sensitivity to LY294002, a small molecule inhibitor of PI3K. Nagata *et al*. (34) provided compelling evidence supporting a role for the PI3K/Akt pathway in trastuzumab resistance. They demonstrated that downregulation of the phosphatase and tensin homolog (PTEN) increased PI3K/Akt signaling and blocked trastuzumab-mediated growth arrest of HER2-overexpressing breast cancer cells. Importantly, using breast tumor biopsies, they showed that an absence of PTEN expression was associated with reduced response to trastuzumab-based therapy. Furthermore, they showed that inhibitors of Akt and mTOR signaling restored trastuzumab sensitivity *in vitro* and *in vivo* (34,43).

More recently, Berns *et al.* (35) identified PTEN as the only gene whose knockdown resulted in trastuzumab resistance using a large-scale RNA interference screen. They followed up this discovery by showing that oncogenic PI3K mutants confer resistance to trastuzumab in cell culture. Most importantly, they demonstrated that oncogenic PI3K mutations or low PTEN expression was associated with reduced trastuzumab response in breast cancer patients, and that combination PI3K mutation and PTEN loss identified those with worst response.

Collectively, these studies strongly support PTEN loss and increased PI3K signaling as predictors of trastuzumab resistance, and suggest that PI3K inhibitors should be explored as potential therapies in patients with trastuzumab-refractory tumors. Clinical trials examining mTOR inhibitors in trastuzumab-refractory populations are ongoing. PI3K inhibitors have been slower to move into the clinic due to multiple PI3K isoforms and concerns about toxicity; however, novel PI3K small molecule inhibitors have recently entered clinical phases of development, and show hope of being tested in trastuzumab-refractory breast cancers.

5.3. Insulin-like growth factor-I receptor cross talk

The first preclinical study to examine trastuzumab resistance demonstrated that stable overexpression of IGF-IR reduced trastuzumab-mediated growth arrest of SKBR3 HER2-overexpressing breast cancer cells (36). In that study, expression of IGF-binding protein 3 (IGFBP3), which blocks IGF-I-mediated activation of IGF-IR, restored trastuzumab sensitivity. In addition, IGFBP3 increased the trastuzumab sensitivity of HER2-stably transfected MCF7 cells, which have high endogenous IGF-IR levels.

By developing an *in vitro* model of resistance by chronic exposure of SKBR3 cells to trastuzumab (28), we further demonstrated that IGF-IR plays a role in resistance. Although total IGF-I receptor levels were unchanged between trastuzumab-sensitive parental cells and resistant cells, a unique interaction between IGF-IR and HER2 was identified exclusively in resistant cells (38). This interaction appeared to facilitate cross talk from IGF-IR to HER2, such that IGF-I stimulation induced phosphorylation of HER2, and inhibition of IGF-IR, either by the neutralizing antibody alpha IR3 or the IGF-IR tyrosine kinase inhibitor I-OMe-AG538, blocked phosphorylation of HER2. This cross talk occurred uniquely in the trastuzumab-resistant cells, and not in the trastuzumab-sensitive parental cells. Furthermore, our results showed that resistant cells exhibit more rapid IGF-I stimulation of downstream PI3K/Akt and MAPK pathways relative to parental cells. Inhibition of IGF-IR signaling either by antibody blockade or IGF-IR tyrosine kinase inhibition restored trastuzumab sensitivity in our *in vitro* resistant model, demonstrating the potential importance of the IGF-I pathway as a therapeutic target in trastuzumab-resistant breast cancer.

In addition, IGF-I stimulation resulted in downregulation of the cyclin-dependent kinase (cdk) inhibiting protein $p27^{kip1}$ in breast cancer cells (37,38). Reduced expression of $p27^{kip1}$ was associated previously with trastuzumab resistance (28,33,44), and increased expression of $p27^{kip1}$ restored trastuzumab sensitivity in our resistant model (28). Hence, $p27^{kip1}$ downregulation may occur subsequent to increased IGF-IR signaling, and reduced $p27^{kip1}$ levels may serve as a predictive marker for trastuzumab resistance.

6. PERSPECTIVE

Cross talk and trans-phosphorylation is an important mechanism utilized by the HER2 receptor to activate multiple signaling pathways and drive mammary tumorigenesis, as well as possibly lead to drug resistance. Interaction and activation of other members of the erbB family may contribute to diminished response to the HER2-targeted antibody trastuzumab. In addition, interaction and cross talk with IGF-IR has been demonstrated in breast cancer cells that have become refractory to trastuzumab (38). HER2/ER cross talk has been shown to result in resistance to endocrine agents in ER+/HER2+ breast cancers (18,19). Thus, a clear understanding of how interactions and cross talk between HER2 and other growth factor receptors impact response to anti-neoplastic therapies can lead to the design and testing of additional targeted agents for use against HER2-overexpressing breast cancers.

A novel HER2-targeted agent, lapatinib (Tykerb, GW572016, GlaxoSmithKline), was recently approved by the U. S. F. D. A. for use against HER2-overexpressing breast cancers in combination with the chemotherapeutic agent capecitabine (45). Lapatinib is a dual inhibitor of the EGFR and HER2 tyrosine kinase domains, and has shown efficacy in preclinical models of HER2-overexpressing breast cancer including trastuzumab-refractory cells (29,40,41). A phase III trial of HER2-overexpressing metastatic breast cancer patients, who were heavily pretreated and trastuzumab refractory, demonstrated that lapatinib plus capecitabine doubled the median time to progression (TTP) and median progression-free survival

(PFS) (both 36.9 weeks) compared with capecitabine alone (median TTP 19.7 weeks and PFS 17.9 weeks) (45).

Lapatinib has been shown to interrupt baseline and ligand-stimulated activity of EGFR and HER2 and to block downstream signaling through the Akt and MAPK pathways *in vitro* and *in vivo* in models of HER2-overexpressing breast cancer (46,47). Published evidence also indicated that lapatinib was able to overcome and inhibit IGF-I cross signaling in trastuzumab-resistant cells (41). Interestingly, a clinical trial of lapatinib in EGFR- or HER2-overexpressing solid tumors demonstrated that clinical response was associated with increased pretreatment expression of phosphorylated and total HER2, MAPK signaling, IGF-IR, p70 S6 kinase (downstream of mTOR pathway), and TGF-alpha compared with non-responders (48). Thus, potential mechanisms leading to trastuzumab resistance may actually increase sensitivity to lapatinib.

In addition to lapatinib, future efforts to further understand the mechanisms and therapeutic implications of interactions of HER2 with other signaling pathways should yield novel therapies for use against breast cancers that have progressed on trastuzumab.

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Abbreviations: HER2, human epidermal growth factor receptor 2; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide-3-kinase; U.S.F.D.A., United States Food and Drug Administration; ER, estrogen receptor; SERM, serum estrogen receptor modulator; TKI, tyrosine kinase inhibitor; HRG, heregulin; AIB1, amplified in breast cancer 1; Src-3, steroid receptor co-activator-3; AI, aromatase inhibitor; TGF, transforming growth factor; PTEN, phosphatase and tensin; IGF-IR, insulin-like growth factor-I receptor; IGFBP, insulin-like growth factor binding protein; cdk, cyclin-dependent kinase; TTP, time to progression; PFS, progression-free survival

Key Words: erbB2, Epidermal Growth Factor Receptor, Insulin-Like Growth Factor-I Receptor, Estrogen Receptor; Review

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