Acquired Resistance to Small Molecule ErbB2 Tyrosine Kinase Inhibitors

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Abstract Breast cancers overexpressing the ErbB2 (HER2) receptor tyrosine kinase oncogene are treated with targeted therapies such as trastuzumab (Herceptin), an anti-ErbB2 antibody, and lapatinib (GW572016/Tykerb), a selective small molecule inhibitor of ErbB2 and epidermal growth factor receptor tyrosine kinases that was recently approved for ErbB2+ breast cancers that progressed on trastuzumab-based therapy. The efficacy of lapatinib as a monotherapy or in combination with chemotherapy, however, is limited by the development of therapeutic resistance that typically occurs within 12 months of starting therapy. In contrast to small molecule inhibitors targeting other receptor tyrosine kinases where resistance has been attributed to mutations within the targeted receptor, ErbB2 mutations have not been commonly found in breast tumors. Instead, acquired resistance to lapatinib seems to be mediated by redundant survival pathways that are activated as a consequence of marked inhibition of ErbB2 kinase activity. For example, inhibition of phosphatidylinositol3 kinase-Akt in lapatinib-treated cells leads to derepression of FOXO3A, a transcription factor that up-regulates estrogen receptor (ER) signaling, resulting in a switch in the regulation of survival factors (e.g., survivin) and cell survival from ErbB2 alone to ER and ErbB2 in resistant cells. In this review, we discuss the effects of lapatinib on signaling networks in ErbB2+ breast cancer cells to elucidate potential mechanisms of therapeutic resistance and strategies to overcome or prevent its development.

Background

Breast cancer is no longer considered a homogeneous disease but rather several subtypes distinguishable by gene expression profiling (1). This has led to a paradigm shift in breast cancer treatment in which targeted therapies are now used to exploit the specific molecular features of individual tumor types. For example, 25% of breast cancers overexpress the ErbB2 (HER2) oncogene tyrosine receptor kinase. These ErbB2$^+$ tumors tend to occur more frequently in premenopausal women and predict for a poor clinical outcome (2). Consequently, ErbB2 represents an attractive target for therapeutic intervention.

ErbB2, a member of the type I transmembrane receptor tyrosine kinase family (ErbB1/EGFR, ErbB2/Her2, ErbB3, and ErbB4), regulates cell growth and differentiation particularly during embryogenesis and breast development during puberty (3). Deregulation of ErbB2 in mammary cells contributes to the development of breast cancer (2).

Binding of soluble epidermal growth factor ligands to their cognate ErbB receptor induces homodimerization or heterodimerization of ErbB2 and autophosphorylation of highly conserved phosphotyrosine residues located within the cytoplasmic domain of the stimulated ErbB receptor (4, 5). These autophosphorylation sites serve as docking sites for adaptor proteins linking ErbB receptors to downstream growth and survival signaling networks (6). ErbB2 is the preferred partner for other ErbB receptors, potentiating the signaling effects of the heterodimeric receptor complex (7). As the only member of the family lacking an exogenous ligand, ErbB2 is transactivated by its heterodimeric partner. Similarly, ErbB3 is the only member of the family that is kinase dead, requiring transactivation by its partner (8). ErbB3 does, however, contain six phosphotyrosine docking sites for the p85 subunit of phosphatidylinositol3 kinase (PI3K; ref. 9), a potent mediator of tumor cell survival and resistance to cancer therapy (10). In breast and other cancers, ErbB2-ErbB3 heterodimers represent one of the most potent prosurvival receptor signaling complexes. Activation of PI3K in turn phosphorylates and activates protein kinase B (Akt), which plays a key role in regulating cell proliferation, apoptosis, glucose homeostasis, cell size, nutrient response, and response to DNA damage (11). The PI3K-Akt signaling pathway is concomitantly up-regulated in ErbB2+ breast cancers, where it exerts prosurvival effects associated with therapeutic resistance (12, 13).

ErbB2 represents an attractive therapeutic target. The standard of care for early and advanced stage ErbB2+ breast cancers is trastuzumab, an anti-ErbB2 monoclonal antibody (14, 15). Although a variety of mechanisms have been proposed (16), the exact mechanism(s) responsible for trastuzumab antitumor activity remain unknown. Preclinical studies do not show consistent down-regulation or inactivation of receptor-linked signaling pathways (17). It now seems that activation of antibody-dependent cellular

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Received 8/13/08; revised 8/14/08; accepted 8/14/08.

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doi:10.1158/1078-0432.CCR-08-0581

cytotoxicity plays a prominent role in trastuzumab antitumor activity (18).

Unfortunately, most advanced stage ErbB2$^+$ breast cancers do not respond to trastuzumab and the majority of responders progress within 12 months of initiating therapy (19). Consequently, other approaches to block ErbB2 signaling have been developed including small molecules that compete with ATP for binding at the catalytic kinase domain of ErbB2(20). Lapatinib (GW572016/Tykerb), an oral, reversible inhibitor of ErbB2 and EGFR tyrosine kinases, was recently approved in combination with capecitabine for treating advanced stage ErbB2+ breast cancers that progressed on prior trastuzumab-based therapies (21). Although lapatinib inhibits both ErbB2 and EGFR tyrosine kinases, its antitumor activity in breast cancer seems to be more dependent on ErbB2 overexpression than EGFR (22, 23). We and others have shown that lapatinib inhibits ErbB2 tyrosine phosphorylation, in turn, inhibiting downstream signaling pathways that regulate tumor cell growth and survival. In addition, Gril et al. (24) recently showed that lapatinib inhibited the formation of brain metastases in a well-established preclinical metastatic breast cancer xenograft model that happens to be resistant to trastuzumab. In this model, inhibition of ErbB2 phosphorylation by lapatinib correlated with the reduction in the size of brain metastases. These findings may have significant clinical relevance because 20% to 30% of women on trastuzumab-based therapies develop brain metastases (25).

Lapatinib also happens to be one of the most specific kinase inhibitors either approved or in clinical development. When the specificity of 20 kinase inhibitors, used at clinically relevant concentrations (≤10 μmoll/L), was compared in an *in vitro* binding assay against a panel of 113 kinases, lapatinib was the most specific, targeting ErbB2, EGFR, and 2 kinases with unknown function (26). Therefore, at concentrations ≤10 μmol/L, the effects of lapatinib on cell signaling are unlikely off-target effects. This level of specificity provides an opportunity to explore the effects of lapatinib on stress responses in ErbB2+ breast cancer cells that may contribute to the development of resistance.

Effects of Lapatinib on Cell Signaling Networks in ErbB2$^+$ Breast Cancer Cells

To understand mechanisms of acquired resistance, it is important to elucidate the effects of lapatinib on complex cell signaling networks in tumor cells. Some of these effects may contribute to tumor cell death, whereas others end up protecting against apoptosis. The following includes a brief summary of the effects of ErbB2 kinase inhibition on cell signaling networks in lapatinib-treated ErbB2+ breast cancer cells:

Mitogen-activated protein kinase. Overexpression of ErbB2 leads to the activation of downstream Ras-Raf-mitogen-activated protein kinase (MAPK)-Erk signaling (27). As we and others have shown, lapatinib inhibits MAPK-Erk1/2 in lapatinib-treated ErbB2+ breast cancer cell lines, tumor xenografts, and in clinical tumor biopsies obtained from women with ErbB2+ breast cancer receiving lapatinib (28). Although MAPK inhibition may play a role in lapatinib antitumor activity, it is clearly not sufficient.

PI3K-Akt. Similar to MAPK, the PI3K-Akt pathway is concomitantly activated in ErbB2$^+$ breast cancers, where it promotes tumor cell survival and resistance to cancer therapies (29). Inhibition of ErbB2 kinase by lapatinib in turns blocks PI3K-Akt signaling in breast cancer cell lines, tumor xenografts, and in clinical tumor biopsies from patients (28). Although the effects of lapatinib treatment on PI3K-Akt seem to contribute to tumor cell death, other unintended consequences of Akt inhibition may activate compensatory prosurvival effects, as discussed below.

Inhibitor of apoptosis proteins. Inhibition of ErbB2, MAPK-Erk, and PI3K-Akt, although perhaps necessary for lapatinib antitumor activity, is not sufficient. Members of the inhibitor of apoptosis protein family are frequently deregulated in cancer, where they contribute to resistance to cytotoxic agents (30). In adults, survivin, the smallest member of the inhibitor of apoptosis protein family, is generally not expressed in nonmalignant cells. In breast cancer, however, survivin expression represents an independent poor prognostic factor predicting for poor clinical outcome (31). Lapatinib, but not trastuzumab, down-regulates survivin in ErbB2$^+$ breast cancer cell lines and in clinical tumors from women treated with lapatinib (32). Survivin down-regulation in lapatinib-treated ErbB2+ breast cancer cells is dependent on PI3K inhibition (Fig. 1) and remains one of the most robust biological correlates of lapatinib antitumor activity (32). In addition, XIAP, another inhibitor of apoptosis protein family member, is inhibited by lapatinib particularly in ErbB2$^+$ inflammatory breast cancer cells where it correlates with induction of apoptosis (33).

Activation of the AMP-regulated kinase metabolic stress response. In studies designed to address the cardiotoxicity associated with trastuzumab therapy, we found that lapatinib, but not trastuzumab, triggered a highly conserved cellular metabolic stress response controlled by AMP-regulated protein kinase (34). Although the exact mechanisms of cardiomyopathy induced by trastuzumab is unclear, ErbB2 inhibition has been found to cause mitochondrial dysfunction in cardiomyocytes (35). The mitochondrial dysfunction results in loss of ATP with consequent contractile dysfunction and cardiomyopathy. GW2974, an analogue of lapatinib, protected cardiac myocytes from apoptotic stimuli by activating AMP-regulated kinase, which in turn switched cell metabolism from an ATP-consuming anabolic state to an ATP-generating catabolic state. Trastuzumab did not activate AMP-regulated kinase and did not protect against an apoptotic stimulus such as tumor necrosis factor α, a proinflammatory cytokine commonly found in cancer patients and cardiac failure patients (34). This may explain in part why lapatinib has a low incidence of cardiotoxicity compared with trastuzumab (36).

GW2974 also activated AMP-regulated kinase and oxidative phosphorylation in ErbB2+ breast cancer cell lines (34). Because tumors are highly dependent on aerobic glycolysis (Warburg effect) as their primary source of energy (ATP) and metabolic intermediates for protein, fatty acid, and nucleic acid biosynthesis (37), the activation of this metabolic stress response and a switch from glycolysis to oxidative phosphorylation on the survival of tumor cells remains to be determined. Because these cells eventually develop resistance to long-term exposure to lapatinib, the assumption is that they activate redundant metabolic pathways as a source of metabolic intermediates for the biosynthesis of macromolecules and generation of ATP.

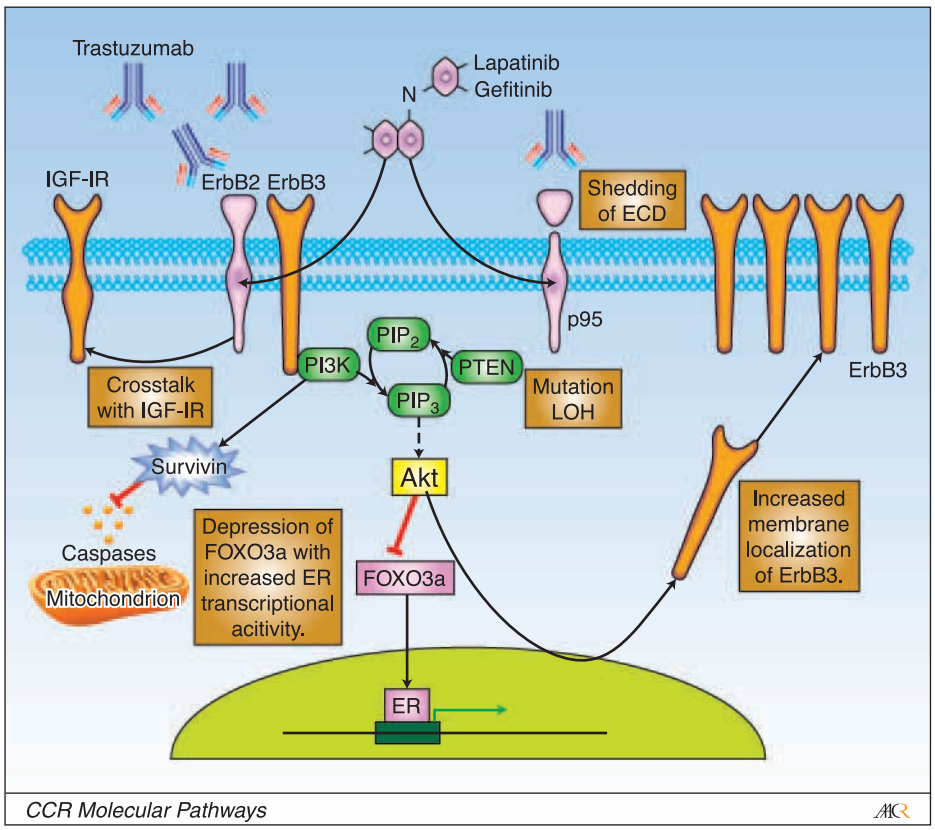

Fig. 1. Proposed mechanisms of acquired resistance: crosstalk with insulin-like growth factor receptor I; PTEN mutation or loss of heterozygosity (*LOH*); depression of FOXO3a with increased ER transcriptional activity; increased membrane localization of ErbB3; extracellular domain (extracellular domain) shedding resulting in p95.

Clinical-Translation Advances

Therapeutic resistance to lapatinib: a clinical dilemma

Most examples of acquired therapeutic resistance to receptor tyrosine kinase inhibitors include development of mutations within the targeted receptors. For example, mutations in BCR/ABL and c-kit confer resistance to a specific BCR/ABL, c-kit kinase inhibitor (imatinib/Gleevec) in chronic myeloid leukemia and gastrointestinal stromal tumor, respectively (38). Strategies to overcome resistance to imatinib as a consequence of these target mutations have been successful, often by improving binding of small molecules to their targets (39, 40). These same strategies may be completely ineffective, however, if resistance has occurred by an alternate mechanism, which seems to be the case in ErbB2-targeted therapies, because mutations within the ErbB2 receptor have not been commonly found in resistant breast tumors (41).

Are mechanisms of resistance to trastuzumab relevant to lapatinib? The answer is probably not. First, lapatinib does not seem to be cross resistant with trastuzumab as lapatinib has clinical activity in ErbB2$^+$ breast cancers that have progressed on trastuzumab-based therapies (21). Second, biological mechanisms attributed to trastuzumab resistance do not seem to apply to lapatinib. These include the following.

Up-regulation of insulin-like growth factor receptor I. Nahta and colleagues (42) showed ErbB2 heterodimerization with insulin-like growth factor receptor I, which restored PI3K-Akt signaling in trastuzumab-resistant breast cancer cell lines. Consequently, inhibition of insulin-like growth factor receptor I induced tumor cell apoptosis. Nahta et al. (42) further showed that an insulin-like growth factor receptor I tyrosine kinase inhibitor inhibited ErbB2 and Akt phosphorylation while inducing p27 (42). Lapatinib disrupted ErbB2-insulin-like growth factor receptor I heterodimers, inhibited ErbB2 and Akt phosphorylation, and induced p27 expression in these trastuzumab-resistant breast cancer cells. When p27 was knocked out, the ability of lapatinib to induce apoptosis was not diminished, suggesting that cell cycle arrest and apoptosis induced by lapatinib was not p27 dependent (43).

p95. Another mechanism enabling ErbB2-dependent breast cancers to evade trastuzumab antitumor activity includes shedding of the extracellular domain of ErbB2 (44). The resulting truncated receptor p95 retains its signaling activity (45). In fact, p95 has been implicated as a poor prognostic factor correlated with increased lymph node metastasis (46). These findings translated into a worse disease-free survival rate compared with breast cancer patients with full-length ErbB2. Because lapatinib does not target the extracellular domain of ErbB2, it is able to inhibit p95 in preclinical models (45, 47). In the clinic, p95$^+$ predicted for a worse outcome compared with breast cancers that only expressed full-length ErbB2 (48).

PTEN-deficient tumors. PTEN, a protein phosphatase with tumor suppressor activity is absent in 50% of breast cancers as a consequence of epigenetic silencing or mutations (49). It has been shown that ErbB2$^+$ breast cancers that are also PTEN deficient respond poorly to trastuzumab (50). PTEN status, however, does not seem to effect lapatinib antitumor activity either in preclinical studies (51) or in women with ErbB2$^+$ breast cancer treated with lapatinib (23).

Up-regulation of ErbB3. To investigate the potential causes of acquired resistance to ErbB2-targeted tyrosine kinase inhibitors, Sergina et al. (52) found that tyrosine kinase

inhibitors only transiently inhibited ErbB3 and Akt despite effective inhibition of ErbB2. Although ErbB2 autophosphorylation was inhibited, there was sufficient residual kinase activity to transphosphorylate and activate ErbB3. Increased ErbB3 sensitivity to reduced ErbB2 kinase activity was achieved by ErbB3 up-regulation. This occurred by increased membrane localization and decreased phosphatase activity toward phosphorylated ErbB3. The forward shift in phosphorylated ErbB3 equilibrium restored PI3K-Akt signaling. As proof of concept, this group was able to abrogate the onset of resistance of breast cancer cells to tyrosine kinase inhibitors with high concentrations of tyrosine kinase inhibitors to ensure near-total ErbB2 kinase inhibition. This feedback mechanism was found to be under the control of Akt (52).

Models of therapeutic resistance to lapatinib

One of the key factors limiting our understanding of the mechanisms involved in lapatinib resistance was the lack of published preclinical models. To address this issue, we developed cell-based models of lapatinib resistance using ErbB2+ breast cancer cell lines that were initially highly sensitive to lapatinib-induced of apoptosis (e.g., IC_{90} < 1 μmol/L). Although treating these cells with lapatinib at clinically relevant concentrations (500 nmol/L-1 μmol/L) initially resulted in significant cell death, continued exposure to lapatinib led to the outgrowth of resistant cells (e.g., rBT474; ref. 53). Resistance did not seem to be associated with loss of ErbB2 expression or sensitivity to lapatinib, as phosphorylation of ErbB2, Akt, MAPK, and ErbB3 were all inhibited in resistant cells (53). What did change was the lack of inhibition of survivin in resistant cells indicating that chronic exposure to lapatinib led to a switch in the regulation of survivin and tumor cell survival from ErbB2 alone to ErbB2 and another redundant survival pathway as discussed below. The development of resistance in these cell lines mimics the clinical setting where women receive lapatinib on a daily, continuous basis, often experiencing dramatic clinical responses lasting for months only to be followed by recurrence and rapid disease progression.

Derepression/activation of a compensatory survival pathway. $ErbB2^+$ breast cancers tend to be either estrogen receptor-negative or low expressers of the estrogen receptor (54). FOXO3a, a transcription factor that promotes estrogen receptor signaling, is suppressed by activated Akt (55), the latter concomitantly up-regulated in $ErbB2^+$ breast cancers. This provides a plausible explanation for estrogen receptor negativity in some $ErbB2^+$ breast cancers. BT474 breast cancer cells constitutively express low levels of estrogen receptor (56) and are therefore not sensitive to antiestrogens such as fulvestrant (53). Inhibition of ErbB2 kinase and downstream inhibition of Akt in lapatinib-treated BT474 cells, however, derepressed FOXO3a, which in turn activated estrogen receptor signaling, thereby switching the regulation of survivin and cell survival from ErbB2 in parental BT474 cells to the estrogen receptor in rBT474 cells (53). Importantly, we showed, in sequential pretreatment and posttreatment clinical tumor, biopsies from women with $ErbB2^+$ breast cancers that lapatinib treatment activated FOXO3a and increased the expression of estrogen receptor and its regulated gene products in some tumors. In fact, these lapatinib-resistant cells were entirely viable despite effective ErbB2 and Akt inhibition (53). These formerly fulvestrant-insensitive breast cancer cells became apoptotic; however, when lapatinib was combined with fulvestrant or complete estrogen deprivation, the latter mimicking the effects of aromatase inhibitors (53). Thus, lapatinib-resistant breast cancer cells do not entirely abandon the ErbB2 signaling pathway, instead developing a codependence between the ErbB2 and ER pathways. Consequently, the combined use of antiestrogens with lapatinib prevented the development of lapatinib resistance in BT474 cells. These findings provided the basis for combined lapatinib and antihormonal treatment, which is currently being evaluated in clinical trials in the neoadjuvant and metastatic settings (57).

Combination therapy

Whether greater clinical benefit to the patient can be achieved by combining therapies upfront to prevent resistance or to treat sequentially maximizing the duration of efficacy of each targeted agent is unknown. Combining lapatinib with capecitabine after progression on trastuzumab improves progression-free survival (21), thus supporting the preclinical finding that lapatinib is able to overcome trastuzumab resistance. In addition, *in vitro* studies have shown synergistic antitumor activity when lapatinib is combined with trastuzumab (58). Clinical trials evaluating this combination are currently ongoing in the advanced stage and neoadjuvant settings. EGF104900 is a phase III trial comparing combination trastuzumab plus lapatinib versus lapatinib alone in metastatic breast cancer patients progressing on trastuzumab-based therapies (59). Patients on this study are heavily pretreated with median pretreatment regimens of four in the lapatinib arm and five regimens in the combination arm. In their preliminary report, investigators reported that the trial reached the primary end point of a 27% reduction in risk of progression with the combination arm (59). At a 6-month analysis, 28% of the combination arm had not progressed versus 13% of the lapatinib monotherapy arm (59). This trial supports the preclinical data showing enhanced antitumor effects with combined lapatinib trastuzumab treatment, highlighting the importance of translational work that combines agents rationally rather than by empiricism.

Sequential therapy and combination therapy involving lapatinib and trastuzumab have thus far shown activity in the metastatic setting. It is too early to say whether one approach is superior to the other.

Conclusions

Breast cancer comprises a heterogeneous group of diseases with complex oncogenic lesions. The use of trastuzumab and lapatinib represents an important advance in breast cancer treatment. The development of acquired resistance, however, poses a formidable clinical challenge to overcome. Empirical combinations of targeted agents are unlikely to yield clinically useful regimens to treat or prevent resistance. As we gain experience with targeted agents both at the bench and clinic and understand how the complex survival pathways interact, we can combine targeted agents rationally.

Disclosure of Potential Conflicts of Interest

N.L Spector: commercial research grant, GlaxoSmithKline; consultant, Array Biopharma. The other authors disclosed no potential conflicts of interest.

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Clin Cancer Res 2008;14:6730-6734.