Gene Amplification of ErbB-2: From Gene to Therapy

Kinnari Pandya and Clodia Osipo

Contents

Abstract

Breast cancer remains the second leading cause of cancer-related deaths worldwide. One of the main obstacles for finding a cure for breast cancer is the inherent heterogeneity of the disease. There are three main subtypes which include estrogen and/or progesterone (ER/PR)-positive, epidermal growth factor receptor-2 (ErbB-2/HER2)-positive, and triple negative that lack expression of ER or PR and express wild type levels of ErbB-2. The etiology of breast cancer development termed the tumorigenic process has been closely linked to gene amplification. Several genes have been shown to be amplified in breast cancer including the ErbB-2 gene on chromosome 17q12-21. The amplification of the ErbB-2 gene is a clear and defined indicator of ErbB-2-positive breast cancer development. In this chapter, we will review the classes of genes that are amplified and linked to breast cancer, discuss the significance of the ErbB-2 signaling pathway to breast cancer progression, targeted therapy, and drug resistance.

1 Gene Amplification: An Oncogenic Driver

Gene amplification refers to duplication of a chromosomal region that contains a gene. It occurs during uneven crossing-over during meiosis between disarranged homologous chromosomes. Multiple different biologically and clinically relevant genes are frequently duplicated or multiplied in breast cancer. Amplification of a gene is one way by which a gene can be overexpressed. The resulting overexpression of a proto-oncogene promotes uncontrolled cell proliferation and drives tumorigenesis by enabling constitutive activation of downstream signaling pathways and by inducing genetic instability, and thus, it usually predicts for poor prognosis. Some studies have shown a correlation between patient survival and number of gene amplifications. In addition, the

K. Pandya · C. Osipo (✉)
Molecular Biology Program, Loyola University Chicago, Maywood, IL, USA
e-mail: cosipo@lumc.edu

C. Osipo
Department of Pathology/Oncology Institute, Loyola University Chicago, Maywood, IL, USA

C. Osipo
Department of Microbiology and Immunology, Loyola University Chicago, Maywood, IL, USA

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function of genes involved plays a critical role in determining tumor characteristics. An early role for gene amplification in the development of breast cancer has been proposed. Gene amplification is a hallmark of malignant transformation and serves as a useful tool in determining targeted therapeutic options and/or prognosis.

The aim of this chapter was, therefore, to provide an overview of oncogenes that are amplified in breast cancer. Particularly, we will focus on understanding the role of ErbB-2 as an oncogenic driver and a therapeutic target in breast cancer.

2 Common Gene Amplifications in Breast Cancer

The following genes have been identified which, when amplified and overexpressed in breast cancer, are associated with high tumor grade, metastasis, poor prognosis, and decreased overall survival: *MYC* (48 %), *PRDM14* (34 %), *TOP2A* (32 %), *ADAM9* (32 %), *HER2* (28 %), *CCND1* (26 %), *EMSY* (25 %), *IKBKB* (21 %), *FGFR1* (17 %), *ESR1* (16 %), and *EGFR* (9 %). Frequently, the chromosomal regions that are amplified with high copy number during breast cancer development include 8p (*FGFR1*, *ADAM9*, *IKBKB*), 11q (*CCND1*, *EMSY*), and 17q (*PPARBP*, *HER2*, *TOP2A*). Most of the common amplifications in estrogen receptor-α (ERα)-positive breast tumors exhibit amplification of 8p and 11q chromosomal regions. However, amplification of the 17q chromosomal region has been identified in both ERα-positive and ERα-negative breast tumors.

Gene amplifications in breast cancer are frequent on chromosome 8p, 11q, and 17q, in which multiple driver oncogenes are amplified independently or together in various combinations. For example, tamoxifen-treated breast cancer patients often exhibit co-amplification of CCND1 and EMSY and this co-amplification predicts for poor survival. Both FGFR1 and CCND1 amplifications were associated with significantly reduced survival. In contrast, simultaneous amplification of HER2 and MYC has been shown to be associated with large tumors, reduced survival, and favorable outcome in response to trastuzumab, an anti-HER2 agent.

Amplification and co-amplification of several genes (oncogenes and tumor suppressors) have been shown to be involved in the development, maintenance, and progression of malignant breast cancer. However, the most comprehensive studies have been conducted in understanding the role of ErbB-2 (HER2/neu) as a proto-oncogene.

3 ErbB-2 Signaling Pathway (Fig. 1)

The human epidermal growth factor receptor-2 (ErbB-2, HER2/neu) is a type I transmembrane receptor tyrosine kinase. ErbB-2 and other family members (EGFR, ErbB-3, and ErbB-4) contain an N-terminal, extracellular ligand-binding domain, a transmembrane domain, and a C-terminal, intracellular tyrosine kinase domain. Unlike the other family members, ErbB-2 is considered to be an orphan receptor as it has no known ligand and ErbB-3 lacks tyrosine kinase activity. Under physiological conditions, ligand binding triggers hetero- or homo-dimerization of ErbB receptors resulting in auto- and transactivation of receptor kinase function. The active receptor tyrosine kinase then triggers various intracellular signaling pathways, including PI3'K and MAPK, resulting in cell survival and proliferation. Not all ErbB dimers exhibit equivalent signaling capacity; homo-dimers transmit weak signals compared to hetero-dimers. As ErbB-2 lacks a ligand, ErbB-2 hetero-dimerizes with other members of the ErbB family. However, the ErbB-2/ErbB-3 is considered the most potent hetero-dimer that promotes breast cancer cell proliferation and disease progression.

4 ErbB-2 Gene Amplification and Overexpression in Breast Cancer

The ErbB-2 proto-oncogene gene is amplified and is considered the main mechanism of ErbB-2 protein overexpression in 20–30 % of invasive breast cancers. ErbB-2-positive breast tumors have poor prognosis and are prone to early and frequent recurrence and metastases. Overexpression of ErbB-2 provides potent and constitutive activation of MAPK and PI3'K signaling pathways to drive breast tumorigenesis. Currently, trastuzumab (Herceptin®), a recombinant, humanized, monoclonal antibody, is a FDA-approved treatment for ErbB-2-amplified breast cancer. Trastuzumab specifically binds the juxta-membrane region of ErbB-2 at the cell surface to inhibit homo- or hetero-dimerization, thereby slowing growth by inhibiting activation and signaling. The best efficacy and positive therapeutic outcome with trastuzumab are observed in women with tumors that overexpress, have amplification, or have high activity of ErbB-2. Trastuzumab showed significant efficacy in the adjuvant settings with an overall response rate of 26 %, which increased to 80 % when combined with chemotherapeutic agents such as taxanes.

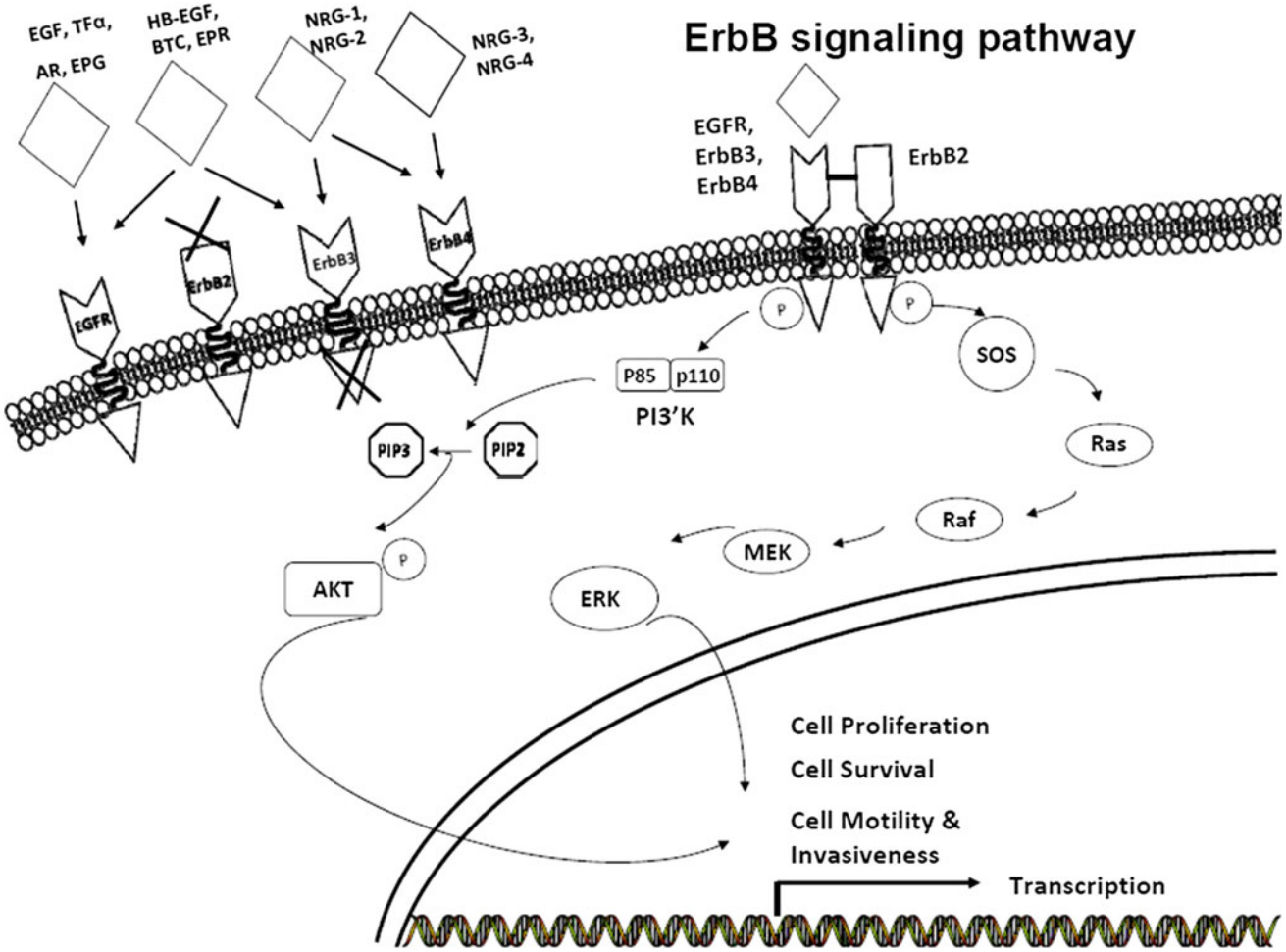

Fig. 1 ErbB signaling pathway

5 Mechanisms of Action (Fig. 2)

The exact mechanism by which trastuzumab inhibits ErbB-2 signaling is not yet fully understood. Some studies have suggested that trastuzumab binds to the extracellular domain of ErbB-2 and upon binding promotes internalization and degradation of ErbB-2 receptor. Other recent studies have demonstrated that trastuzumab selectively blocks ErbB-2/ErbB-3 hetero-dimerization. In addition, binding of trastuzumab to ErbB-2 blocks cleavage of the extracellular domain of the receptor, resulting in decreased levels of constitutively active and soluble p95-ErbB-2. As a result, trastuzumab acts as a potent anti-proliferative and anti-survival agent. Trastuzumab is also capable of inducing immune responses such as antibody-dependent cellular cytotoxicity (ADCC) against ErbB-2-overexpressing tumor cells. The Fc domain of trastuzumab engages with Fc receptors on immune effector cells (T cells) leading to lysis of tumor cells that overexpress ErbB-2. Furthermore, trastuzumab has anti-angiogenic effects. Trastuzumab initiates one important cellular response and that is cell cycle growth arrest in G1 phase, which is often accompanied by decrease in cyclin D1 levels and an increase in p27 levels. Trastuzumab induces little if any apoptosis.

6 Mechanisms of Resistance

Although trastuzumab has had a tremendous impact on improving survival for women with ErbB-2-positive breast cancer, trastuzumab resistance remains a major problem, particularly with metastatic tumors. Unfortunately, 66–88 % of women with metastatic breast cancer are resistant to trastuzumab as a single agent. Furthermore, many of the women who initially respond to trastuzumab-based treatments that include chemotherapy develop resistance within the first year of treatment. Approximately 15 % of women who receive trastuzumab will develop recurrent breast cancer, which almost always is metastatic to distant organs and ultimately results in death. In women with trastuzumab-resistant disease,

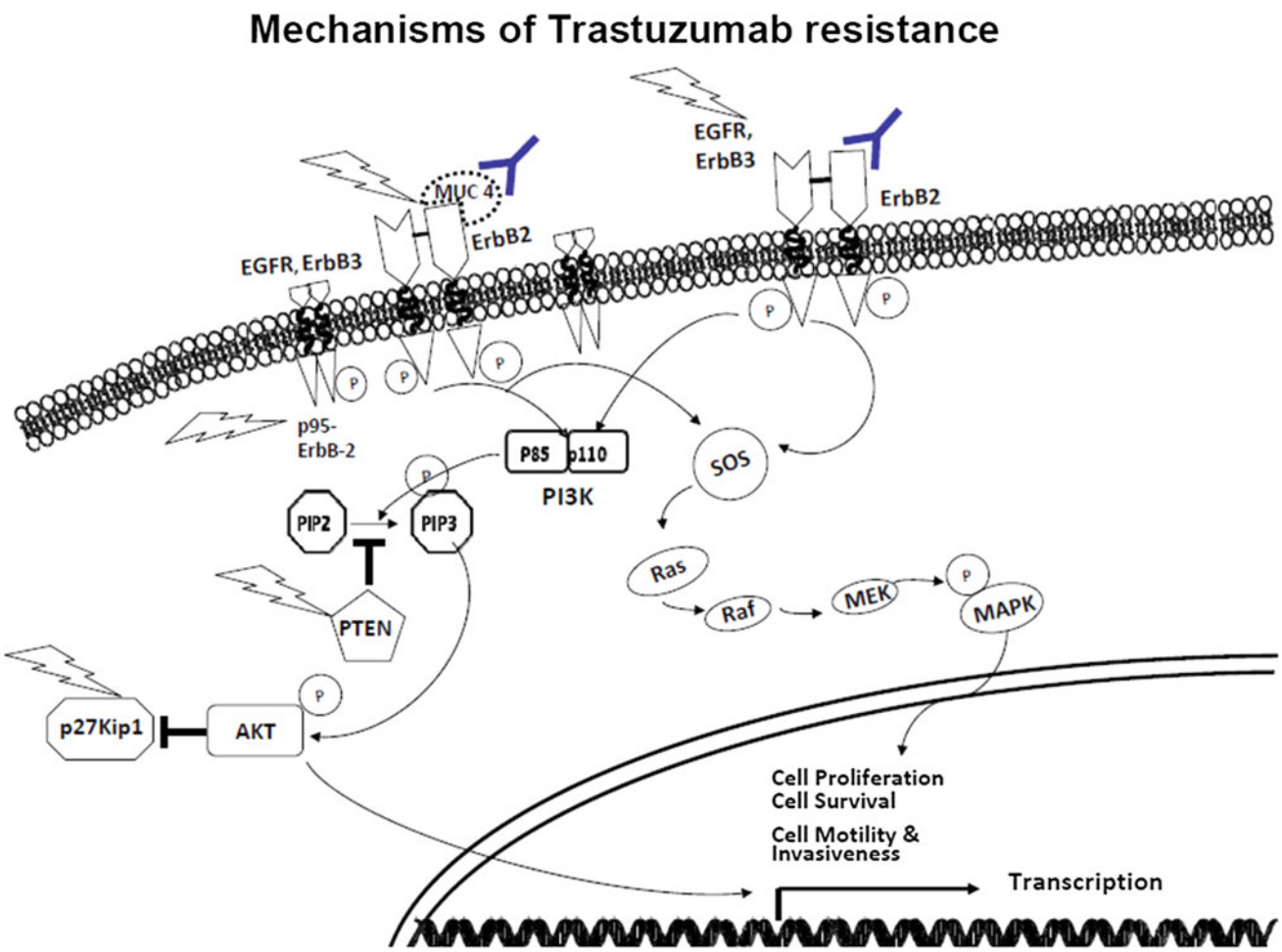

Fig. 2 Mechanisms of trastuzumab resistance

lapatinib, a small molecule, dual EGFR/ErbB-2 tyrosine kinase inhibitor (TKI), has been clinically proven to overcome some resistance to trastuzumab. However, resistance to lapatinib has been observed in patients within the first year of treatment. Thus, despite initial efficacy in the treatment of metastatic disease with anti-ErbB-2 agents, resistance occurs with no clinical means currently available to circumvent it. Thus, understanding the mechanisms responsible for resistance to ErbB-2-targeted therapies is critical to identify novel targets.

6.1 Functional Redundancy Among ErbB Family Members

ErbB family members are functionally redundant. The critical functions of ErbB signaling include dimerization, tyrosine phosphorylation, and activation of some redundant downstream signaling molecules. Even though trastuzumab inhibits ErbB-2 phosphorylation, it rarely blocks the dimerization of ErbB-2 with other ErbB family members. Recently, long-term trastuzumab treatment of ErbB-2-positive breast cancer cells showed increase in EGFR and ErbB-3 expression. This indicates that alternate ErbB family dimers, such as ErbB-1/ErbB-1 and ErbB-1/ErbB-3 dimers, could possibly circumvent trastuzumab-induced blockade and promote growth and survival of breast tumors. Moreover, TGF-β has been shown to activate ErbB-3 in ErbB-2-overexpressing cells and subsequently the PI3'K pathway by enhancing phosphorylation and translocation of ADAM17 to the cell surface. This results in an increase in ErbB ligand shedding and desensitization of these cells to trastuzumab. Interestingly, from EGFR and ErbB-3 receptor knockdown studies, ErbB-3 has been shown to play a crucial role over EGFR in ErbB-2-amplified breast cancer. Therefore, a promising approach to treat trastuzumab resistance would be to design monoclonal antibodies that can be directed at dimerization of all the ErbB family members. Pertuzumab is a humanized, monoclonal antibody that was designed to specifically target hetero-dimers of the ErbB family. Recently, pertuzumab has shown significant efficacy when combined with trastuzumab in ErbB-2-positive metastatic breast cancer.

6.2 Role for Loss of Negative Regulators

Loss or decreased expression of negative regulators of signaling pathway activated by ErbB receptors has been implicated in resistance to trastuzumab. For example, the tumor suppressor phosphatase and tensin homolog (PTEN) is a negative regulator of the PI3'K/AKT signaling pathway. PTEN acts as a phosphatase to dephosphorylate PIP3 back to PIP2. This dephosphorylation results in inhibition of AKT pathway and subsequently controls cell survival, proliferation, and growth. ErbB-2-overexpressing breast tumors that express little to undetectable levels of PTEN respond poorly to trastuzumab therapy. Concurrently, constitutive PI3'K/AKT kinase activity has also been shown to promote growth and proliferation of breast tumors. ErbB-2-overexpressing breast cancer cells that have heightened PI3'K/AKT signaling and reduced PTEN expression were shown to be the most sensitive to PI3'K or mTOR inhibitors. These inhibitors when combined with trastuzumab were able to overcome resistance both in vitro and in vivo breast tumor models. These results suggest that loss or low expression of PTEN and subsequent high AKT kinase activity induce trastuzumab resistance and serve as predictors of poor response to trastuzumab. Thus, inhibitors of the PI3'K/AKT/mTOR signaling pathway need to be explored in combination with trastuzumab to prevent trastuzumab resistance. However, when tumor samples from trastuzumab-treated women were analyzed for PTEN and AKT status, the expression levels of PTEN and AKT did not significantly correlate with response to trastuzumab-based therapy, time to disease progression, or incidence of CNS metastases.

As described above, trastuzumab induces cell cycle growth arrest in G1 phase, which is often accompanied by an increase in a critical negative regulator of cell cycle progression, p27. Loss of expression of $p27^{Kip}$ has been implicated in trastuzumab resistance. The $p27^{Kip1}$ binds to cyclin E either alone or in a complex with cyclin-dependent kinase 2 (Cdk2) and inhibits the catalytic activity of Cdk2 to prevent Cdk2 from adding a phosphate group to its substrate. Trastuzumab induces a G1 cell cycle arrest within the breast tumor by enhancing the association of $p27^{Kip1}$ with cyclinE/Cdk2 complexes, thus increasing the half-life of $p27^{Kip1}$ and preventing phosphorylation of $p27^{Kip1}$ by Cdk2 and subsequent ubiquitin-dependent degradation. Decreased $p27^{Kip1}$ levels and increased Cdk2 levels have been reported in trastuzumab-resistant breast cancer. Depletion of $p27^{Kip1}$ using either antisense or siRNA prevented trastuzumab-induced growth inhibition in ErbB-2-positive breast cancer cells. Conversely, overexpression of $p27^{Kip1}$ or preventing $p27^{Kip1}$ degradation using a proteasome inhibitor MG132 resensitized resistant cells to trastuzumab. These results suggest that $p27^{Kip1}$ could be yet another crucial marker of trastuzumab resistance. However, $p27^{Kip1}$ protein expression has yet to predict response to trastuzumab-based therapy in patients with ErbB-2-overexpressing, metastatic breast cancer.

6.3 Accumulation of Soluble p95-ErbB-2

The efficacy of trastuzumab to inhibit ErbB-2 depends on its ability to recognize the juxta-membrane epitope of ErbB-2 and bind with high avidity. However, full-length ErbB-2 is a substrate for ADAM metalloproteinases and has been reported that a soluble form of ErbB-2 is detectable in serum of breast cancer patients. The remaining truncated version of ErbB-2 (p95) lacks the critical trastuzumab binding site within the extracellular domain. The p95-ErbB-2 can dimerize with other family members in a ligand-independent manner and constitutively turn on downstream signaling pathway. Approximately 30 % of ErbB-2-amplified breast cancers exhibit p95-ErbB-2 expression and is associated with adverse outcome and resistance to trastuzumab. Breast cancer cell lines (expressing low levels of ErbB-2) transfected with p95-ErbB-2 showed sensitivity only to lapatinib, whereas transfection with full-length ErbB-2 exhibited sensitivity to both trastuzumab and lapatinib. A retrospective analysis of 46 patients confirmed that expression of p95-ErbB-2 increased tumor growth and led to trastuzumab resistance, whereas expression of wild-type ErbB-2 maintained sensitivity to trastuzumab. These data suggest that tumors expressing constitutively active p95-ErbB-2 maintain their dependence on ErbB-2 activity for proliferation and may respond better to alternative approaches to inhibiting ErbB-2.

6.4 Role for MUC4: Altered Receptor–Antibody Interaction

Trastuzumab exerts its anti-tumor activity by binding and inhibiting ErbB-2 at the cell surface. Thus, altering the interaction between ErbB-2 and trastuzumab could serve as an emerging mechanism that could contribute to trastuzumab resistance. For example, MUC4, a membrane-associated mucin, functions by modulating ErbB-2 signaling. The ascites sialoglycoprotein-2 (ASGP-2) subunit of glycoprotein MUC4 directly interacts with ErbB-2 via an EGF-like domain, masking ErbB-2 and inhibiting trastuzumab binding to ErbB-2. Elevated MUC4 expression is observed during acquired trastuzumab resistance. This interaction was associated with increase in phosphorylation of ErbB-2 at tyrosine 1248, which plays a major role in ErbB-2-driven tumorigenesis. MUC4 activates ErbB-2, without affecting the expression of ErbB-2. Inhibition of MUC4 using siRNA

increased trastuzumab binding and sensitized resistant breast cancer cells to trastuzumab. Thus, novel agents targeting MUC4 expression and/or function in combination with trastuzumab might prove to be advantageous in the treatment of resistant tumors.

6.5 Crosstalk Between ErbB-2 and Notch Signaling Pathways

Recently, Notch signaling has emerged as a target for the treatment of breast cancer. Notch-1 is another breast oncogene and a potent cell fate receptor. Women diagnosed with breast cancer that co-overexpress Notch-1 and its ligand Jagged-1 have the poorest overall survival. Notch-1 and Notch-4 are breast oncogenes that promote breast cancer tumorigenesis by simultaneously inhibiting differentiation, promoting survival, and proliferation. We have identified, Notch-1, as a novel target in trastuzumab-resistant breast cancer. Based on our recent findings, we showed that ErbB-2 inhibits Notch-1 activity. We showed that when breast cancer cells that overexpress ErbB-2 are treated with trastuzumab, the unintended consequence is activation of Notch-1. This increased Notch-1 signaling decreased the effectiveness of trastuzumab. We recently showed using preclinical xenograft models that simultaneous inhibition of Notch and ErbB-2 significantly decreased recurrence of ErbB-2-positive breast tumors and reversed trastuzumab resistance.

7 Alternative Treatment Options for ErbB-2-Overexpressing Breast Cancer

Despite the advances that have been made by trastuzumab and lapatinib, patients with metastatic breast cancer develop resistance to anti-ErbB-2 agents during the course of treatment and eventually develop disease progression. The table below shows alternative treatment strategies for patients with resistant breast cancer.

ErbB-2 dimerization inhibitor	Pertuzumab
ErbB-2 ADCC	T-DM1
PI3'K inhibitor	LY294002
Tyrosine kinase inhibitors	Lapatinib, neratinib, BIBW 2992
mTOR inhibitors	Everolimus
HSP90 inhibitors	Tanespimycin
VEGF receptor inhibitors	Bevacizumab
IGF-1R inhibitors	NVP-AEW541, CP-751871
Notch pathway inhibitors	GSI

8 Conclusions

Overexpression of ErbB-2 as a result of gene amplification has provided an outstanding opportunity to develop targeted therapy for breast cancer. It has for the most part been a very successful example of how identification of oncogene amplification has led to an FDA-approved treatment strategy. Trastuzumab has had a tremendous impact on improving survival for women with ErbB-2-positive breast cancer. However, resistance to trastuzumab remains a major problem, particularly among women with metastatic disease. Thus, elucidating the molecular mechanisms underlying intrinsic or acquired anti-ErbB-2 drug resistance may provide crucial information about patients that fail to respond to therapy or develop resistance within the first year of their treatment. Thus, there is an immediate urge for genomic, transcriptomic, and proteomic approaches to better understand the mechanisms of ErbB-2-targeted drug resistance. Some of the molecular mechanisms for acquired resistance and possibly intrinsic resistance summarized in this book chapter include overexpression of redundant ErbB family members, overexpression of MUC4, and loss of negative regulators (PTEN and $p27^{Kip1}$). We have identified a novel biomarker of trastuzumab resistance: Notch-1. Compensatory increase in Notch-1 activity upon trastuzumab treatment could provide a survival advantage to breast cancer cells, driving tumorigenesis, and resistance. Activated Notch-1 may contribute to resistance by regulating previously identified molecular markers of trastuzumab resistance, activating alternative signaling pathways, and potentiating crosstalk between the tumor and its surrounding microenvironment. Thus, a thorough analysis of the role of Notch signaling in ErbB-2-amplified breast cancers would provide an evidential rationale of whether targeting the Notch pathway could improve the way trastuzumab-resistant ErbB-2-positive breast cancer patients are treated today.

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