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Resistance to HER2-targeted Therapy

Reza Valadan [1], Alireza Rafiei [1*], Mohsen Tehrani [1], Forough Nejatollahi [2]

[1] Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
[2] Recombinant antibody laboratory, Department of Immunology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

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Corresponding Author:
Alireza Rafiei
Department of Immunology, Molecular and Cell Biology Research Center, Faculty of Medicine, KM 17 Khazarabad Road, Khazar Sq, Sari, Iran.
Phone:+98-113543614
Email: Rafiei1710@gmail.com

Abstract

Production and approval of trastuzumab (Herceptin®) for the treatment of metastatic breast cancer (MBC) was a millstone in antibody-based targeted therapy in the cancer treatment. However, despite the early success in the clinical trials, trastuzumab failed to appreciate the initial attraction due to development of resistance to the drug. Majority of patients who benefit from the drug acquire resistance to it and experience tumor recurrence within 1 year. Several molecular and cellular mechanisms underlying the resistance to trastuzumab have been proposed. In this review, first, we provide a brief history leading to production of trastuzumab. Also we consider the cellular and molecular antitumor effects of trastuzumab and then, we discuss the mechanisms underlying trastuzumab resistance in four levels.

Keywords: Trastuzumab; ErbB; Breast cancer; Mechanisms of action; Resistance

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Introduction

Epidermal growth factor receptor family (ErbB family) is a group of transmembrane receptor tyrosine kinase that is essential for normal cell growth and development. They sense extracellular stimuli and signal it to nucleus through tightly regulated signaling pathwaysthat result into general and cell-specific responses (1-3). The ErbB family consists of four homolog receptors: epidermal growth factor receptor EGFR/ErbB1 (HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) (4). Generally, they compromise a tethered extracellular ligand binding domainin their resting state, a single pass transmembrane domain, and intracellular tyrosine kinase. The extracellular ligand-binding domain is made up four homologous subdomains designating domains I-IV or L1, CR1, L2 and CR2, respectively (5). In normal cells, upon ligand binding to the extracellular domain, a conformational rearrangement occurs in the extracellular domain that exposes the dimerization domain (domains II or CR1).This ligand-mediated rearrangement induces homo and heterodimerization of these receptors (2-3). Dimerization is a fundamental step in the subsequent signaling pathways that is followed by receptor auto-phosphorylation and trans-phosphorylation leading to recruitment of downstream signaling cascades. These downstream signaling pathways end with a variety of cellular and molecular responses including cell growth and death, proliferation, differentiation, migration, an production of vascular endothelial growth factor (VEGF) (3,6) . Although the general structures and functions of the four members of ErbB family are the same, some variations exist in the types of ligands and in the way they activate. ErbB1 and ErbB4 have active tyrosine kinase domains and known ligands, while ErbB3 can bind several ligands but lacks intrinsic tyrosine kinase domain. In contrast, ErbB2 is an orphan receptor with no yet identified ligand (7-8). No need to ligand binding, ErbB2 can adopt an extended (open) active conformational state (resembling to ligand-activated state) that is readily available for dimerization (8-9) .Neither ErbB2 nor ErbB3 can lead to signaling by itself, thus receptor heterodimerization is essential for their function. Heterodimerization of the orphan ErbB2 with a kinase-defective ErbB3 accounts for potent receptor pairingand efficient signaling in which, ErbB3 provides ligand-binding domain and ErbB2is responsible for intracellular signal transduction. This is supported by evidence that ErbB3 is preferred

heterodimeraztion partner of ErbB2, also it is the most transforming dimer in fibroblasts and her2 overexpressing breast cancer cell lines (10-12). However, in the case of HER2 positive breast cancers, there is an overexpression of ErbB2 on the cell surface that favors simultaneous homo-and heterodimerization of the receptor without absolute need for a ligand (3).

Essentially, all combinations of receptor homodimerization and heterodimerization are possible in the ErbB family, providing a network of signaling pathways that diversifies and amplifies signals in different cell types and different micro-environments (3). On the other hand,these diverse signaling pathways are thought to be responsible for failure of ErbB-targeted therapy in cancer patients, notably, in Her 2 positive breast cancer patients. In this review, first,we focus on the mechanisms underlying resistance to trastuzumab, one of the most well-known drugs for HER2-targeted therapy, in breast cancer patients and then, we consider the hierarchical network of ErbB receptor family in response to trastuzumab.

Avian erythroblastosis virus and rodentHER2/ NeugenelinkedErbB receptors to oncogenesis

Early studies in the 1980s, revealed that avian erythroblastosis virus encodes an oncogene which is a truncated form of EGRF missing the extracellular domains while containing an active tyrosine kinase domain. This protein is responsible for dys-regulated growth and tumorgenesis of infected cells in chicken (13). Subsequently, it was shown that a variant of Her2 receptor in rat, encodes HER2/Neu oncogene containing a valine to glutamic acid substitution in the transmembrane domain of the receptor. Although this point mutation results in the constitutive activation of tyrosine kinase domain in rat, such mutation has not ever been reported in human (14). Unlike the rat HER2/Neu, human Erbb2/HER2 undergoes various degrees of gene amplification in some cancers (15). Transforming ability of overexpressed ErbB2 in human and rat fibroblasts has been experimentally evaluated and demonstrated that high levels of the ErbB-2 expression was associated with malignant transformation of NIH/3T3 cells. This is completely in consistence with the condition of human mammary gland tumors overexpressing HER2 (16-17). Following these initial findings, direct roles of EGFR and ErbB2 receptors in cancer development and progression were discovered in several human malignances (18-19). EGFR and ErbB2 undergo various types of alternation including gene amplification, protein overexpression, and point mutations and deletions in human cancer (15,20-22).Recently, potential roles of dys-regulated and mutant variants of ErbB3 and ErbB4 in cancer initiation and development were reported in several human cancers (23).Although, a role for Erbb4 receptor in cancer is less clear, some studies reported somatic mutations in the kinase domain of ErbB4 receptor which were linked to development of malignant melanoma, while other studies indicated that ErbB4 may function as a tumor suppressor in breast and prostate cancer (23-24).

Anti-HER2 therapy: From bench to bedside

Soon after the discovery of ErbB2 involvement in breast cancer and several others, it attracted the attention of pharmacological companies and scientists all over the world to find strategies that might block ErbB2 signaling in cancer cells. Although several theoretical and experimental treatment strategies have been described and reviewed elsewhere (19, 25). To date, two main approaches have found their ways to clinic, first, the production of monoclonal antibodies directed against ErbB2/HER2 ectodomain in order to disrupt HER2 signaling and second, specific inhibition of intracellular tyrosine kinase domain by small chemical compounds(26-27).Trastuzumab (Herceptin; Genentech, USA) was the first drug to be approved by FDA in 1998 to use for the treatment of HER2 positive breast cancer. Trastuzumab is a humanized monoclonal antibody directed against the jaxtamembrane subdomain (CR2/IV) of ErbB2 extracellular domain (9). It was first raised in mouse immunized with NIH3T3/HER2 cell line expressing HER2with routine hybridoma technology (26). Preclinical data revealed that the parental antibody of trastuzumab (4D5) showed a range of anti-proliferative and cytotoxic effects against invasive breast tumor cell overexpressing ErbB2 in vitro. Furthermore, trastuzumab significantly reduced the resistance to the cytotoxic effect of TNF-α (27). However, 4D5 could not be directly administrated in humans due to production of anti-mouse antibody response in the body. Therefore, antibody humanization, that is a process in which, the complementary-determining regions (CDRs) of murine antibody is grafted to a human IgG construct, was inevitable. First attempts to humanize 4D5 antibody failed, since the resulting antibody did not block the proliferation of human breast carcinoma SKBR3 cells. Additional minor amino acid changes were needed in the CDRs of 4D5 to enhance the antibody affinity and function that eventually entered trastuzumab into clinical trials (28). Phase III clinical trial of trastuzumab along with chemotherapy in metastatic breast cancer (MBC) patients resulted in a longer time to disease progression (median, 7.4 vs. 4.6 months;), a higher rate of objective response (50 percent vs. 32 percent), a longer duration of response (median, 9.1 vs. 6.1 months), a lower rate of death at 1 year (22 percent

vs. 33 percent), longer survival (median survival, 25.1 vs. 20.3 months) and a 20 percent reduction in the risk of death (29).

Trastuzumab shows a range of cellular and molecular antitumor effects

To date, various mechanisms by which Trastuzumab inhibits tumor cell growth and invasion were proposed. However not fully understood, binding of trastuzumab to the ErbB2 was shown to induce various molecular and cellular effects including cell cycle arrest, induction of apoptosis, cytotoxic targeting by immune cells and molecularly, inhibition of DNA repair and downregulation VEGF.

ErbB2 is common regulator of cell growth and development in normal epithelial cells that activates two main intracellular signaling pathways including RAS-MAPK and phosphatidylinositol 3'-kinase (PI3 kinase) pathways resulting in cell growth and inhibition of cell death, respectively (3). Trastuzumab binds to the extracellular domain of ErbB2 and is believed to inhibit intracellular signaling pathways through decreasing receptors phosphorylation, increasing $p27^{Kip1}$ levels and interaction with CDK2, decreasing CDK2 activity (30), increasing membrane localization of PTEN and decreasing phosphorylated Akt levels and activity (31-32), that collectively resulted in cell cycle arrest and induction of apoptosis. Shedding of ErbB2 extracellular domain from cell surface is responsible for constitutive activation of tyrosine kinase domain and downstream signaling pathways (33). In patients treated with trastuzumab and docetaxel there was a decrease in serum level of the receptor ectodomain indicating that trastuzumab might stabilize receptor integrity or prevent receptor proteolysis (34-35). Downregulation of HER2 on the cell surface was seen in the SKBR3 and MDA453 cells treated with trastuzumab in vitro, providing another mechanism of action through receptor endocytosis and degradation. However, it is unclear whether diminished receptor signaling is resulted from trastuzumab-mediated internalization of the receptor or it may directly downregulate receptor expression on the cell surface (36).Importantly, trastuzumab targets HER2 overexpressed cancer cell to cytotoxic effects by immune cells. Antibody dependent cell mediated cytotoxicity (ADCC) is a potential mechanism of cancer regression in the trastuzumab treated patients. In preoperative patients and mice cancer model, there were large infiltrations of macrophage and natural killer cell in the cancer tissue following treatment with trastuzumab (37). Natural killer cells and macrophages, bearing Fc gamma receptor (FCγ) on their cell surface can recognize FC domain of trastuzumab bond to the cancer cells, therefore facilitating the lysis of cancer cells. Mice xenograft tissue overexpressing HER2 as a cancer model, showed 90% and 96% in tumor regression following treatment with 4D5 and trastuzumab, respectively In contrast, FCγ receptor deficient mice ($Fc\gamma R^{-/-}$) could not provoke strong cytotoxic activity that only 20 and 44% tumor mass reduction were seen in the trastuzumab and 4D5 treated mice (38). Similar results were obtained from in vitro ADCC assays on multiple cancer cell lines (39).

One of the most important consequences of HER2 signaling is expression and production of vascular endothelial growth factor (VEGF) accordingly; HER2 overexpression in cancer cells is highly associated with increased VEGF expression, angiogenesis, and invasiveness of the tumor (40). Trastuzumab may prevent angiogenesis in the invading cancer cells by downregulation of VEGF (41). Overall results obtained from trastuzumabin the clinical trials were satisfactory at the time especially when combined cytotoxic chemotherapy drugs leading to drug approval by FDA in 1998.

Trastuzumab at work: trastuzumab failed to appreciate the initial attraction

Although trastuzumab has been approved for the treatment of metastatic breast cancer and adjuvant therapy in HER2 overexpressing breast cancer, the majority patients who show initial response to the treatment acquire resistance to trastuzumab and experience tumor recurrence less than a year later. Although, molecular mechanism underlying the resistance to trastuzumab is poorly defined, several potential mechanisms were proposed based on both in vitro and in vivo studies. Here, we describe the mechanisms of resistance to trastuzumab in four levels.

Level 1: Changes in HER2-Trastuzumab interaction

I) Shedding of HER2ectodomain

Shedding of HER2 extracellular domain remains a truncated membrane-bound fragment, p95 on the cell surface that is believed to have more tyrosine kinase activity compare to the intact HER2 (33). Although trastuzumab may prevent shedding of HER2 ectodomain and subsequent production of constitutively active p95, HER2 overexpressing cancer cell undergoes a slow proteolytic cleavage of extracellular domain. Tumor cells containing p95 are selected and proliferated over the course of the disease therefore HER2 signaling pathways continued even in the presence of trastuzumab. Treatment of p95-expressing cell lines or MCF-7p95HER2 xenograft tumors with trastuzumab had no effect on cell growth rate and growth inhibition

indicating that intact HER2 structure is necessary for anti-proliferative action of trastuzumab. Similarly, small percentage of patients expressing p95 responded partially to trastuzumab therapy (1 out 46 patients), whereas 19 of the 37 patients (51.4%) with tumors expressing full-length HER2 achieved either a complete (five patients) or a partial (14 patients) response (42).

On the other hand, large amount of sheded HER2 extracellular domain may compete with membrane-bound HER2 for binding to trastuzumab on the cancer cells. These circulatory trastuzumab-HER2 complexes undergo faster blood clearance compared with trastuzumab alone thus decreasing the drug bioavailability in the blood stream (43). Phase II study of weekly administration of anti-p185HER2 monoclonal antibody in patients with HER2/Neu-overexpressing metastatic breast cancer showed that higher plasma concentrations of HER2 extracellular domain were associated with shorter serum half-life of anti-p185HER2 antibody (44).

II) Muscin4 and CD44/hyaluronan polymer complex may interfere with trastuzumab binding to the receptor

Another potential mechanism of resistance to trastuzumab is mediated through aberrant expression of MUC4 on the surface cancer cells. MUC4 is a large membrane-associated glycoprotein, expressed largely in normal epithelial tissues,including mammary gland, uterus, colon, cornea and trachea. MUC4 is also overexpressed or aberrantly expressed on a number of human tumors including breast tumors (45). MUC4 expression in cancer cells is thought to mask trastuzumab binding epitope on HER2 extracellular domain, leading to diminished binding of trastuzumab to the receptor. JIMT-1, an experimental model of trastuzumab-resistance cell line, which is established from a breast cancer patient showing HER2 gene amplification and primary resistance to trastuzumab, provided insights how aberrant expression of a receptor may interfere with antibody binding to HER2. Although, the expression profile of HER2 and trastuzumab-induced HER2 internalization in JIMT-1 cells were similar to those in trastuzumab-sensitive lines, the expression of MUC4 was higher in JIMT-1 than in trastuzumab sensitive cell lines. In addition, the level of MUC4 expression was inversely correlated with the trastuzumab binding capacity in this cell line. Downregulation of MUC4 expression in JIMT-1 by RNA interference (RNAi) restored trastuzumab sensitivity and binding to these cells, indicating that steric hindrance induced by MUC4 may interfere with the interaction of trastuzumab to its cognate epitope on the receptor. Unexpectedly, it is also demonstrated that overexpression of MUC4 prevent natural interaction of HER2 with its dimerization partners, as the level of phosphorylated tyrosine kinase domain of HER2 were lower in JIMT-1 cells compare to those in trastuzumab sensitive cell lines (46).

Binding of hyaluronan to CD44 forms a receptor/ligand complex on the cell surface that is supposed to limit the access of trastuzumab to HER2receptor, leading to drug resistance. It has been shown that JIMT-1 cell lines not only express high level of MUC4 but also express a significant amount of CD44 and the level of CD44 expression correlates with level of ErbB2 downregulation in vivo. Furthermore, RNAi studies revealed that trastuzumab-induced internalization of HER2 is dependent on CD44 expression on the cell surface. On the other hand, it was demonstrated that CD44/hyaluronan polymer complex hindered the access of trastuzumab to the receptor. To address this problem, 4-methylumbelliferone (4-MU), a hyaluronan synthase inhibitor, has been used to increase the efficiency of trastuzumab-mediated antitumor effects. Following treatment of severe combined immunodeficiency (SCID) mice bearing JIMT-1 xenografts with 4-MU, a decline in pericellular hyaluronan concentration aroundJIMT-1 cells was observed, leading to increased binding of trastuzumabto HER2. Similar results were obtained from in vitro studies in which 4-MU treatment of JIMT-1 cell line enhanced the amount of bound trastuzumab relative to the intensity of HER2 (47).

III) HER2 mutations may provide another mechanism of resistanceto trastuzumab

Disrupted receptor-antibody interactions may potentially originate from mutations in the HER2 extracellular domain and decline in HER2 expression levels over the course of treatment. One might postulate that somatic mutations in the HER2 extracellular domain occur in a small subset of trastuzumab-resistant patients, preventing from effective recognition and binding of antibody to receptor. Although such mutations have not been reported in the HER2 extracellular domain in breast cancer patients, several somatic mutations in the tyrosine kinase domain were found in a small percentage of invasive ductal mammary carcinomas (4/3 %), gastric carcinomas (5%), and colorectal carcinomas (2.9%) (48).These mutations along with mutations in intracellular signaling pathway proteins may contribute to resistance to intracellular tyrosine kinase inhibitors.

Level2: changes in the intracellular signaling pathway proteins and adaptor proteins

I) Phosphatase and tensin homolog (PTEN) deficiency

HER2 overexpression in breast cancer patients

initiates a cascade of intracellular phosphorylation events,leading to cell transformation and oncogenesis. Initially, PI3K (Phosphoinositide 3-kinase) is activated by dimerized HER2 and is followed by generation of phosphoinositide and translocation of AKT to plasma membrane. Phosphorylated and activated AKT in turn, phosphorylates numerous targets (3). In trastuzumab-responsive patients, PTEN, the negative regulator of AKT phosphorylation, is localized to cell membrane where it dephosphorylate membrane phosphatidylinositol - 3,4,5 triphosphate (PI3,4,5P3), preventing recruitment of AKT to the cell membrane and reducing growth promoting signals (31). PTEN is a potential tumor suppressor factor and PTEN deficiency has been linked to nearly 50%of breast cancers and many others. PTEN deficiency due to loss of function mutations and transcriptional and epigenetic alternation of PTEN is believed to play important roles in tumor formation and resistance to trastuzumab in breast cancer patients (31, 49). It was demonstrated that PTEN downregulation by antisense oligonucleotide in *in vitro* and *in vivo* tumor models contributed to trastuzumab resistance and increased in PI3K signaling, by contrast, restoration of PTEN activity in PTEN deficient cell lines inhibited AKT activation and tumor formation (31,50). PTEN activity is a powerful predictor of trastuzumab response in breast cancer patients and PTEN deficiency is associated with lower overall response rates to trastuzumab and poorer prognosis in the patients. It has also been suggested that PI3K inhibitors could be considered in the therapeutic regimes of PTEN-deficient patients in order to overcome trastuzumab resistance since PI3K inhibitors rescued trastuzumab resistance in PTEN-deficient cells in vitro and in vivo (31).

II) Modulation of $p27^{kip1}$

Induction of $p27^{kip1}$ and reduction of CDK2 is associated to trastuzumab-mediated cell cycle arrest and growth inhibition in HER2 overexpressing breast cancer patients. Upregulation of $p27^{Kip1}$during cell cycle G1 phase, as one of the most important cyclin-dependent kinase (CDK) inhibitor, induce cell cycle arrest while, its downregulation causes cell growth promotion and proliferation and has been linked to many types of human tumors including breast cancer (51,52). It has been suggested that proteasomal degradation of $p27^{Kip1}$ via ubiquitin-dependent pathway is a potent mechanisms of trastuzumab resistance in breast cancer patients. This is supported by evidence that downregulation of $p27^{Kip1}$ by small interfering RNA reduced trastuzumab-mediated growth arrest of HER2-overexpressing SKBR3 breast cancer cells (51). Additionally, trastuzumab-resistant patients showed lower $p27^{kip1}$ expression levels and higher cyclin-dependent kinase 2 CDK2 activity compared to trastuzumab-responsive patients. Importantly, exogenous induction of $p27^{Kip1}$ to resistant cells or inhibition of proteasomal degradation of $p27^{Kip1}$ by the proteasome inhibitor MG132 restored trastuzumab sensitivity to resistant cells, suggesting that trastuzumabresistancemay be associated with decreased $p27^{kip1}$ levels in breast cancer cells.

Level 3: failure in antibody-mediated cytotoxicity

Antibody dependent cell mediated cytotoxicity (ADCC) accounts for a potential antitumor property of trastuzumab and any failure in each step of this process lead to the drug ineffectiveness. It was documented that natural occurring variants of human FcγRIIIA (158V/F) showed different affinities of IgG1 to Fc receptors. PBMCs and natural killer cells isolated from FcγRIIIa V/V donors mediated better ADCC activity in cells treated with trastuzumab and anti-HER2 IgG1 than those carrying the F allele (FcγRIIIaF/F) (53). In a study conducted by Musolino et al, fifty-four HER-2 positive breast cancer patients treated with trastuzumab plus taxane for metastatic breast cancer were evaluated in the respect to FcγRIIIa-158V/F polymorphisms. They showed that the FcγRIIIa-158 V/V genotype was significantly correlated with objective response rate (ORR) and progression-free survival (PFS). The ADCC analysis showed that PBMCs isolated from patients bearing V/V alleles had a significantly higher trastuzumab-mediated cytotoxicity than PBMCs harboring different genotypes (54).

Level 4: interfering and compensatory roles of alternative signaling pathways

As it has been proposed by yarden (3), ErbB receptors are organized into a richly interactive, multilayered network, that are not only affected by their own ligands and interactions but also are stimulated by heterologous signals, including hormones, neurotransmitters, lymphokines and stressinducers. This network of networks is responsible for tightly regulated general and cell-specific biological responses throughout growth and development. On the other hand, these complex networks provide an opportunity for cancer cells to escape from anti-HER2 monotherpy.

I) Trastuzumab-mediated ErbB2 blockade is compensated by other ErbB receptors

Recently, critical roles of EGFR and ErbB3 in triggering of MAPK and PI3K signaling pathways have been highlighted in some studies. Although HER2 signaling is thought to be partially blocked in

the presence of trastuzumab, trastuzumab has little or no effects on EGFR and HER3 signaling. Therefore cancer cells that coexpress either of these receptors pair may initiate PI3K and MAPK signaling even in the presence of trastuzumab resulting in failure of treatment. EGFR and ErbB3 are frequently expressed in human mammary tumors along with ERBB2. Experimental evidence showed that in ovariectomized nude mice, heregulin (HRG) the well-recognized ligand for ErbB3, promoted metastasis and preneoplastic transformation of the mammary tissue. It was shown that aggressive phenotype of HRG may be mediated via an increase in activated MAPK, an increase in a matrix-degrading enzyme, and the overexpression of vascular endothelial growth factors (55).By contrast downregulation of HRG with antisense RNA in MDA-MB-231 cells resulted in suppression of the aggressive phenotype of MDA-MB-231 breast cancer cells by inhibiting cell proliferation, preventing anchorage-independent growth in vitro and marked reduction in tumor formation, tumor size, and lack of metastasis in vivo (56).

A subset of BT-474 of breast cancer cell line with increased trastuzumab resistance has been recently described by Ritter et al (57). These cells overexpress HER2 along with EGFR, transforming growth factor alpha, heparin-binding EGF, and heregulin compared with the parental trastuzumab-sensitive cells. They exhibited higher levels of phosphorylated epidermal growth factor receptor (EGFR) and EGFR/HER2 heterodimers in the presence of trastuzumab suggesting that heterodimerization of HER2 with EGFR accounts for a potential oncogenic unit that may not be disrupted with trastuzumab. The importance of dual inhibition of ErbB receptors has been well-documented by Lapatinib (Tykerb/Tyverb, GSK). It is EGFR and HER2 dual tyrosine kinase inhibitor that showed significant responses in the treatment of trastuzumab-resistant patients. Patients with high with high levels of EGFR, HER-2, downregulate pAKT and pERK responded to tyrosine kinase inhibitor not to trastuzumab (58). Similarly, a more recent anti-HER2 antibody pertuzumab, that has been designed to disrupt HER2 homodimerization and heterodimerzation with other ErbB receptors, emphasized the importance of dual or pan-ErbB approaches in the treatment of breast cancer. conclusively, it was proposed by Britten (24) that the most efficient ErbB-targeted strategies to treat ErbB-expressing tumor are (a) combinations of agents that target individual ErbB receptors, (b) single agents that target multiple ErbB receptors, and (c) agents that interfere with ErbB receptor interactions (24).

II) Cross-talk signaling of IGFR1 and HER2

Insulin- like growth factor-I receptor (IGFR1) is another transmembrane tyrosine kinase receptor that is usually co-express with ErbB receptors in breast cancer. Binding of IGFs to IGFR1 results in receptor autophosphorylation and recruitment and phosphorylation of Src homology and IRS-1 adaptor protein. Similar to HER2, IGFR1 activates MAPK and results in cell proliferation and inhibition of cell death (59). Coexpression of IGFR1 and her2 was associated to resistance to trastuzumab in breast cancer cell lines, SKBR3 and MCF-7/HER2. MCF-7/HER2 and SKBR3 cell heterogeneously overexpressed IGFR1 were resistance to anti proliferative effects of trastuzumab, while parental cells showed massive cell death in the presence of trastuzumab. However, the addition of IGF binding protein-3, which decreased IGF-IR signaling, restored trastuzumab-induced tumor cell death (60).

It was shown that following IFGR1 stimulation, IGFR1 physically interacted with HER2 and subsequently phosphorylated it only in trastuzumab resistance cell line not in parental trastuzumab sensitive cell line. Interestingly, inhibition of IGFR1 tyrosine kinase domain with I-OMe-AG538 decreased HER-2 phosphorylation and increased sensitivity of resistant cells to trastuzumab. Furthermore, blocking of IGFR1 with anti-IGFR1 antibody α-IR3 restored sensitivity to trastuzumab suggesting that IGFR1/HER2 heterodimerization occurred in trastuzumab resistance cell line (61). Huang et al (62) extended their study to consider the potential role of ErbB3 in IGFR1/HER2 heterodimer. They observed that heterotrimeric complex formed by erbB2, erbB3, and IGF-IR, enhanced activation of downstream signaling pathways in trastuzumab-resistant breast cancer cells. Downregulation of ErbB3 and IGFR1 by short hairpin RNA in these cells resulted in upregulation p27kip1, inactivatation downstream receptor signaling, and restoration of trastuzumab sensitivity indicating the potential importance of alternative signaling pathways as therapeutic targets for trastuzumab-resistant breast cancer.

Conclusion

The importance of trastuzumab in the treatment of HER2 positive breast cancer patients has been well-documented and emphasized elsewhere. However, targeting HER2 with trastuzumab alone does not seem sufficient to completely kill cancer cells. Cancer cells use a complex overlapping network of signaling pathways to guarantee their survival and growth. Importantly, cooperative and compensatory roles of ErbB receptors family members in complexity of HER2-targeted therapy have been recently reported in breast cancer. Therefore, current approaches in the treatment of HER positive breast cancer are directed to develop dual or pan-ErbB

therapies. As it is exemplified by recent FDA approval, pertuzumab, a novel anti-HER2 antibody specifically inhibit HER2 homo and heterodimerization with other ErbB receptors. Although, combination therapy of trastuzumab and pertuzumab with other chemotherapy drugs may further increase the efficacy of treatment, the experience with trastuzumab has revealed the need for deep understanding of ErbB receptor biological behaviors.

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