Anti-HER2 Cancer Therapy and Cardiotoxicity

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Abstract: A significant milestone in the treatment of breast cancer is the identification of the HER2 receptor as a drug target for cancer therapies. Trastuzumab (Herceptin), a monoclonal antibody that blocks the HER2 receptor, is among the first of such drugs approved by the US Food and Drug Administration for targeted cancer therapy. Clinical studies have shown that Trastuzumab significantly improves the overall survival of breast cancer patients. However, an unforeseen significant side-effect of cardiotoxicity manifested as left ventricular dysfunction and heart failure. Concurrent studies have demonstrated the essential role of the HER2 receptor in cardiac development and maintaining the physiological function of an adult heart. The HER2 receptor, therefore, has become a critical link between the oncology and cardiology fields. In addition to Trastuzumab, new drugs targeting the HER2 receptor, such as Lapatinib, Pertuzumab and Afatinib, are either approved or being evaluated in clinical trials for cancer therapy. With the concern of cardiotoxicity caused by HER2 inhibition, it becomes clear that new therapeutic strategies for preventing such cardiac side effects need to be developed. It is the intent of this paper to review the potential cardiac impact of anti-HER2 cancer therapy.

Keywords: HER2, Cardiotoxicity, Cancer, Therapy.

INTRODUCTION

The HER2 receptor tyrosine kinase is overexpressed in about 25-30% of metastatic breast cancer and is a predictor of poor prognosis [1]. Trastuzumab, a monoclonal antibody that directly binds and blocks HER2, significantly improves cancer therapeutics and overall survival in breast cancer patients [2]. Unexpectedly, the incidence of New York Heart Association class III/IV heart failure is increased nearly five-fold (16 vs. 3 %) when Trastuzumab was used concurrently with anthracyclines compared to anthracyline alone [2]. This clinical finding along with early developmental biology studies in mice have opened a new research field on HER2 signaling in the adult heart which led to the discovery of Neuregulin1 (NRG1) as a new therapeutic approach for the treatment of heart failure [3-9].

A decade of research has demonstrated that the HER receptors and NRG1 are crucial for maintaining mutiple aspects of cardiac physiological function and cell survival, especially in the presence of cardiac stress, such as doxorubicin, hypertension, myocardial infarction, and viral infection [10, 11]. Mechanisms have been suggested for the cardioprotective effects of the NRG1-HER signaling which include inhibiting cardiomyocyte apoptosis and oxidative stress; maintaining cardiomyocyte myofibril structure and calcium homeostasis; promoting cardiomyocyte proliferation and stem cell differentiation into the cardiac lineage [7, 12-18].

In addition to Trastuzumab, US FDA approved Lapatinib and Pertuzumab for anti-HER2 therapy. (www.fda.gov). Drugs targeting multiple HER receptors, such as MM-111, Afatinib and Neratinib are now being tested in clinical trials (www.clinicaltrials.gov). These drugs inhibit HER2 via mechanisms different from Trastuzumab by either targeting a different extracellular domain of HER2 or inhibiting HER2 tyrosine kinase activity [19-29]. Combination therapies of Trastuzumab with Lapatinib or Trastuzumab with Pertuzumab are also in practice [19]. A main purpose of developing drugs targeting multiple HER receptors or combined use of drugs targeting different domains of the HER2 receptor is to overcome the drug resistance of single agents [30, 31]. With a broader spectrum of inhibition, these drugs or therapeutic strategies could cause more severe cardiac side-effects. Lapatinib, a HER2 receptor tyrosine kinase inhibitor, however, causes fewer cardiac side effects than Trastuzumab in patients, suggesting that cardiac outcome may also be dependent on the chemical profile of each drug [32]. Here we review the cardioprotective effects of the HER2 receptor and discuss the potential cardiac impact of anti-HER2 cancer therapy.

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THE HER2 RECEPTOR

The HER2 receptor belongs to the epidermal growth factor receptor family which includes HER1 (also known as epidermal growth factor receptor-EGFR), HER2 (also known as ErbB2 or Neu), HER3 (ErbB3), and HER4 (ErbB4) [33]. At least 11 ligands of the HER receptors are identified [33]. A unique feature of the HER receptor family is that there are no known ligands for the HER2 receptor while the HER3 receptor lacks strong intrinsic tyrosine kinase activity; However, the HER2 and HER3 receptors can form a potent mitogenic receptor complex [34, 35]. The HER receptors are type I transmembrane proteins which are composed of an extracellular region, a single membrane spanning region and a cytoplasmic tyrosine kinase region. The extracellular region contains four domains (I/L1, II/CR1, III/L2 and IV/CR2) [34]. Crystal structures of ligand bound HER1 and HER3 receptors suggest that without ligand binding, domain II interacts with domain IV, exhibiting a "closed" form of receptor; Upon ligand binding to domains I and III, the HER1 and HER3 receptors are transformed to an "open" form, freeing domain II (from IV) to mediate receptor dimerization [33, 34, 36]. The HER2 receptor, however, exhibits a fixed conformation that resembles a ligand-activated state, suggesting the HER2 receptor is "primed" to form dimers with other HER receptors [33, 36].

Upon ligand binding, HER receptors form homo- or heterodimers, followed by phosphorylation of tryrosine residues on the receptors [33, 36]. The HER receptors then recruit adaptor proteins to the phosphorylated docking sites within the intracellular domains. This sets into motion a complex cascade of downstream signaling consisting of two canonical pathways, the phosphoinositide 3-Kinase (PI3K) and the mitogen-activated protein kinase (MAPK), both of which promote cell proliferation, prevent apoptosis and increase tumor angiogenesis and metastasis [33].

1381-6128/14 $58.00+.00

DRUGS TARGETING THE HER2 RECEPTOR AND MECHANISM OF ACTION

The HER2 receptor is overexpressed in about 25-30% of metastatic breast cancers and is associated with poor prognosis [1, 37]. In 1998, the US FDA approved the first anti-HER2 drug Trastuzumab (Herceptin, Genentech) for the treatment of HER2 positive metastatic breast cancer [38]. Since then, several anti-HER2 agents have been approved for breast cancer therapy including Lapatinib (Tykerb, GlaxoSmithKline, 2007), Pertuzumab (PERJETA™, Genentech, 2012) and Ado-Trastuzumab emtansine (T-DM1, Kadcyla, Genentech, 2013) [39-41]. In addition, more drugs are being evaluated in clinical trials for HER2 positive metastatic breast cancers and Trastuzumab pretreated disease [42, 43]. These agents include MM-111 (Merrimack Pharmaceuticals), Afatinib (BIBW2992; Boehringer Ingelheim), and Neratinib (Puma Biotechnology/Pfizer). Recently, novel human anti-HER2 immunoagents are in development, which show lower cardiotoxicity in preclinical animal models (Table **1** and Fig. **1**).

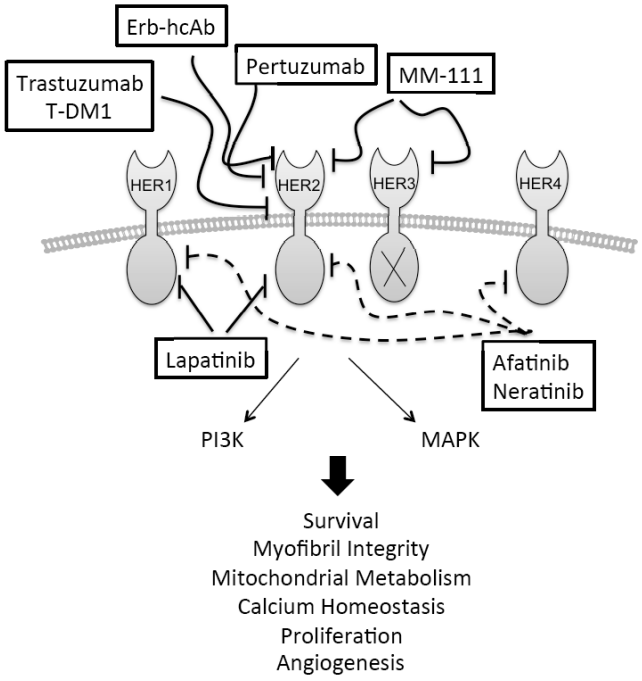

Fig. (1). HER signaling in the heart and drugs targeting HER receptors. The HER receptor tyrosine kinases (HER1, HER2, HER3 and HER4) are pivotal for maintaining the physiological function of an adult heart. Upon ligand binding, the HER receptors form homo- or hetero- dimers, followed by phosphorylation of tyrosine residues on the receptors, and activation of various signaling pathways in the cell, including the PI3K and MAPK pathways. The activation and integration of these signaling pathways lead to the regulation of key cell functions, such as survival, mitochondrial metabolism, proliferation, maintaining myofibril structure and calcium homeostasis in cardiomyocytes, as well as angiogenesis. The HER receptors are drug targets for cancer therapy. Drugs targeting the HER receptors include: Trastuzumab, a monoclonal antibody that targets the domain IV of the HER2 receptor; Pertuzumab, a monoclonal antibody that targets the domain II of the HER2 receptor; Erb-hcAb, a compact anti-HER2 antibody targets the domain I of the HER2 receptor; MM-111, a bispecific antibody that targets both HER2 and HER3; Lapatinib, a reversible kinase inhibitor that targets HER1 and HER2; as well as Afatinib and Neratinib, irreversible kinase inhibitors that target HER1, HER2 and HER4.

Trastuzumab

Trastuzumab is a humanized monoclonal antibody. It binds to the domain IV of the extracellular region of the HER2 receptor [44]. Trastuzumab inhibits HER2 positive breast cancer growth via several proposed mechanisms which include activation of antibody-dependent cellular cytotoxicity (ADCC) [45]; facilitation of the internalization and degradation of the HER2 receptor and subsequent exposure of HER2 epitopes, leading to the lysis of tumor cells by circulating T lymphocytes [46, 47]; inhibition of HER2 extracellular domain cleavage and formation of membrane bound HER2 p95 fragment [48]; inhibition of PI3K and MAPK pathways; enhancement of apoptosis; activation and stabilization of phosphatase and tensin homolog deleted on chromosome 10 (PTEN); increase of cell cycle arrest via upregulating $p27^{Kip1}$ and inhibition of tumor angiogenesis [49-52].

Lapatinib

Lapatinib is a small molecule inhibitor of HER1 and HER2. It works intracellularly by directly targeting the tyrosine kinase domain [53]. Lapatinib binds to the cytoplasmic ATP-binding site of the kinase and blocks receptor phosphorylation and activation of PI3K and MAPK pathways. This leads to an increase in apoptosis and decrease of cellular proliferation [54, 55].

Pertuzumab

Pertuzumab is a recombinant humanized monoclonal antibody (2C4) and is the first in a new class of agents called HER dimerization inhibitors (HDIs) [20, 56]. Pertuzumab binds to the extracellular domain II of the HER2 receptor; therefore, inhibiting homo- or hetero- dimerization of HER receptors and IGF1R [20]. Pertuzumab is approved in combination with Trastuzumab and Docetaxel in patients who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease [42].

Ado-Trastuzumab Emtansine (T-DM1)

Ado-Trastuzumab Emtansine (T-DM1) is an immunoconjugate consisting of the monoclonal antibody Trastuzumab and an antimicrotubule cytotoxic agent mertansine (DM1) [41]. When T-DM1 binds to the HER2 receptor, a propotion of the receptors are thought to be internalized by endocytosis; this is followed by the intracellular release of active DM1, which in turn exerts anti-tumor effects [57, 58].

MM-111

MM-111 is a bispecific antibody that targets the HER2 and HER3 expressing tumors. The HER2 arm is responsible for initial tumor targeting and docking, while the HER3 arm is designed to block Neuregulin (Heregulin) - induced signaling [59].

Afatinib

Afatinib (BIBW 2992) is an oral irreversible small molecule inhibitor of EGFR (HER1), HER2 and HER4 [60]. It is an anilinoquinazoline derivative that is designed to covalently bind to Cys 773 of EGFR and Cys 805 of HER2 [25, 61].

Neratinib

Neratinib is a small molecule irreversible receptor tyrosine kinase inhibitor with activity against HER1, HER2 and HER4 [62, 63]. It prevents autophosphorylation of the receptor by forming a covalent bond with the conserved cysteine residue of the ATP-binding pocket within the kinase domain of the receptor.

Erb-hcAb

In addition to Herceptin, novel human anti-HER2 immunoagents are in development. These include immunoRNases (a human single-chain antibody fragment (scFv) fused with a human RNase) and compact antibodies (two human scFv molecules fused with the Fc region of a human IgG1). Erbicin-human compact antibody (Erb-hcAb) is a human compact anti-HER2 antibody that consists of the anti-ErbB2 (HER2) scFv Erbicin fused to CH2, CH3 and

Table 1. Drugs targeting the HER2 receptor for cancer therapy

Name	Category	Targets	Sponsor	References
Trastuzumab (Herceptin)	Monoclonal antibody	HER2 – domain IV	Genentech	[44]
Lapatinib (Tykerb)	Kinase inhibitor	HER1, HER2	GlaxoSmithKline	[53]
Pertuzumab (PERJETA)	Monoclonal antibody	HER2 – domain II	Genentech	[20]
Ado-Trastuzumab Emtansine (KADCYLA)	Monoclonal antibody linked to the cytotoxic agent mertansine	HER2	Genentech	[41]
MM-111	Bispecific antibody	HER2, HER3	Merrimack Pharmaceuti-cals	[59]
Afatinib (Gilotrif)	Irreversible Kinase inhibitor	HER1, HER2, HER4	Boehringer Ingelheim	[60]
Neratinib (HKI-272)	Irreversible Kinase inhibitor	HER1, HER2, HER4	Puma Biotechnol-ogy/Pfizer	[63]
Erb-hcAb	Compact anti-HER2 antibody	HER2		[64]

hinge regions of a human IgG1 [64]. Compared to Herceptin, Erb-hcAb exhibits several new features: (1) Erb-hcAb is a reduced size antibody. It lacks the CH1 and CL domains of a full size immunoglobulin, yet it preserves all functionally relevant antibody regions. Its size is smaller than Herceptin (100 vs. 155 kD), (2) Erb-hcAb recognizes domanin I of the HER2 receptor, which is different from Herceptin, and (3) Preclinical studies show that compared to Herceptin, Erb-hcAb has less cardiotoxic effects in terms of cardiomyocyte survival and it does not block NRG1 mediated survival signals in cardiomyocytes. On the other hand, Erb-hcAb is effective for inhibiting growth of several human breast cancer cell lines [65, 66].

Combination therapies for overcoming drug resistance

One of the emerging challenges with anti-HER2 therapy is drug resistance [67]. The rate of primary resistance to Trastuzumab used as a single agent is 66-88%, with resistance develops within the first year [68]. Similarly, drug resistance is found in Lapatinib treated breast cancer patients [69]. In addition, nearly 80% of patients pretreated with Trastuzumab failed to respond to Lapatinib [70].

Various mechanisms of Trastuzumab resistance have been proposed which include: (1) Failure of binding to its target HER2. Trastuzumab targets the extracellular domain of HER2. In HER2 positive tumors, the extracellular domain of the HER2 receptor can be proteolytically cleaved and released into circulation. A truncated 95 kilodalton HER2 protein remains bound to the cell membrane with constitutively active tyrosine kinase activity and can form functional p95HER2-HER3 heterodimers [71]. In addition, increased expression of proteins such as mucin-4 (MUC4) and CD44 may mask HER2 and disrupt the binding of Trastuzumab to the HER2 receptor [72]. Another possibility is that the mutations in the HER2 receptor may prevent Trastuzumab recognition and binding to the receptor [73]; (2) Activation of HER1 and HER3 receptors. Overexpression of HER1 or HER3 receptors and their ligands, as well as increase of other growth signals such as insulin-like growth factor-1 (IGF1), Neuregulin (Heregulin), epidermal growth factor (EGF) and transforming growth factor-α (TGFα) [74-76]; (3) Activation of the PI3K pathway. Activation of the PI3K pathway is one of the mechanisms of Trastuzumab resistance. This can be caused by a deficiency of PTEN [77], a phosphotase that inhibits PI3K activity by converting PtdIns(3,4,5)P3 to PtdIns(4,5)P2, or mutation of PIK3CA, the gene encoding PI3Kp110α. It can also be caused by activation of alternative growth pathways, such as insulin-like growth factor1receptor (IGF1R) [76, 78].

Based on these findings, combination therapies have either been approved by the US FDA or are in clinical trials to overcome drug resistance to single agents. These combination therapies include combined use of Trastuzumab, Lapatinib and Paclitaxel, or combined use of Trastuzumab, Pertuzumab and Docetaxel [79, 80]. At the same time, tyrosine kinase inhibitors targeting a broader range of HER receptors, such as Afatinib and Neratinib, have been developed for this purpose [43]. A common feature of second-generation tyrosine kinase inhibitors is irreversible inhibition (Laptinib is reversible). This allows for permanent elimination of kinase activity until a new receptor is synthesized, which can provide prolonged suppression of the target, which is likely necessary to achieve maximal antitumor activity [62].

ANTI-HER2 THERAPY INDUCED CARDIOTOXICITY: CLINICAL FINDINGS

Trastuzumab: Trastuzumab has consistently been shown to have a clinical benefit for the treatment of HER2 positive breast cancer. The therapy also has been found to have adverse cardiac effects. Cardiotoxicity was not observed in the initial phase II trials [81, 82]. In the study by Slamon, *et al*. 63 out of 464 patients were identified as having symptomatic or asymptomatic cardiac dysfunction [2]. The most affected patients were those receiving anthracyclines (AC), in conjunction with Trastuzumab, with a 27% incidence reported in this group versus 8% in patients receiving AC alone. The toxicity was also observed in the group receiving paclitaxel: 1% receiving chemotherapy alone versus 13% receiving chemotherapy and Trastuzumab. The incidence of patients categorized as having New York Heart Association Class III or IV cardiac dysfunction also varied based on the subgroup: 16% received AC

plus Trastuzumab, 3% received AC alone, 2% received paclitaxel and Trastuzumab, and 1% received paclitaxel alone.

The cardiotoxicity reported in the pivotal Trastuzumab clinical trials led to the development of an independent Cardiac Review and Evaluation Committee (CREC). The CREC was created to retrospectively analyze seven phase II and III Trastuzumab trials and provide an unbiased assessment of the risk of cardiac dysfunction associated with Trastuzumab [83]. Four criteria for a diagnosis of cardiac dysfunction (CD) were determined: (1) cardiomyopathy with a decrease in left ventricular ejection fraction (LVEF) either global or more severe in the septum, (2) symptoms of congestive heart failure (CHF), (3) signs of CHF including S3 gallop and tachycardia, (4) asymptomatic decline in LVEF by 10% to less than 55% or a decline in LVEF by 5% to less than 55% with signs or symptoms of CHF. The CREC reviewed 1,219 records and found 112 patients met criteria for CD. The majority of patients (n=83) experienced symptoms and all but one of the symptomatic patients received treatment for CHF. The treatments implemented included diuretics, angiotensin converting enzyme inhibitors, cardiac glycosides, and inotropic agents. The CREC determined that 79% of patients improved with treatment. Because of these findings, subsequent Trastuzumab clinical trials were designed to avoid concurrent use of anthracyclines and Trastuzumab, and include close monitoring of cardiac symptoms and function [83].

Telli *et al*. reviewed Trastuzumab-related cardiotoxicity in major clinical trials, which include National Surgical Ajuvant Breast and Bowel Project (NSABP) B-31, North Central Cancer Treatment Group (NCCTG) N9831, Herceptin Ajuvant (HERA), Breast Cancer International Research Group (BCIRG) 006, the Finland Herceptin (FinHer), and Programme Adjuvant Cancer Sein (PACS) 04 trials [84, 85]. In these trials, Trastuzumab was administered following completion of AC or all chemotherapy, alone, or with paclitaxel or docetaxel. Cardiac function was monitored by either echocardiography or multiple-gated acquisition scan (MUGA).

These studies show that the rate of cardiac death and severe congestive heart failure were not significantly different between Trastuzumab and non-Tratuzumab treated patients (<4%), yet a greater number of patients discontinued Trastuzumab due to asymptomatic decline of cardiac function. A long-term follow up study showed that although cardiac function partially recovered right after cardiac treatment in most patients (80% patients in HERA trial), a significant number of patients (17 in 59 patients) who initially recovered had decline of LVEF to less than 50% during follow up (range from 2.5 to 51.6 months) after reaching acute recovery [86]. This finding suggests that the initial concept regarding the reversibility of Trastuzumab induced cardiac dysfunction needs to be reconsidered [85]. A recent published study further identified additional risk factors that increase the incidence of Trastuzumab-induced heart failure, which include old age (>80 years), coronary artery disease, and hypertension [87, 88]. Studies are ongoing to identify biomarkers that may be used for early detection of Trastuzumab-induced cardiotoxicity, such as cTnI [84, 88].

Lapatinib: Early trials suggested that Lapatinib therapy did not carry with it similar cardiac dysfunction risks as Trastuzumab [89, 90]. Perez *et al* reviewed cardiac safety data in 44 clinical trials. Lapatinib monotherapy was administered in 3689 patients in the studies and 1301 patients served as controls. LVEF was prospectively evaluated by MUGA or echocardiograms at screening and every 8 weeks during therapy and at withdrawal. They analyzed cardiac events defined as symptomatic or asymptomatic with a LVEF decrease greater than or equal to 20% relative to baseline and below the institution's lower limit of normal. Of the study patients, only 1.6% (n = 60) experienced a cardiac event and of those patients only 0.2% (n = 7) were symptomatic. The important finding in the study is that the rate of cardiac events was similar to those that did not receive Lapatinib [89, 90].

There are several proposed mechanisms of the difference in cardiotoxicity between Trastuzumab and Lapatinib. First, Trastuzumab is a monoclonal antibody which induces antibody dependent cell mediated cytotoxicity (ADCC) in HER2 positive breast cancer cells. It could induce ADCC against cardiomyocytes; whereas Lapatinib, a tyrosine kinase inhibitor, dose not produce this effect. However, this theory is not supported by clinical findings. No increase of mononuclear cells in myocardial biopsies has been found from patients who developed Trastuzumab induced heart failure extra-cellular domain [89]. Pertuzumab, another monoclonal antibody targeting the excellulardomain of the HER2 receptor induces fewer cardiac events than Trastuzumab [91]. Second, although both Trastuzumab and Lapatinib target the same receptor, they may trigger different intracellular signaling responses in cardiomyocytes, partially due to off-target effects of Lapatinib [92]. It is shown that Lapatinib activates AMPK (AMP-activated protein kinase) whereas Trasuzumab inhibits AMPK in cardiomyocytes. AMPK is a pivotal energy sensor. It promotes ATP production and maintains energy homeostasis. Cardiomyocytes heavily rely on ATP for maintaining contractile function. It is likely that the less cardiotoxicity induced by Lapatinib is related to its off-target effect on activating AMPK [93]. Third, the better cardiac safety data seen in Lapatinib (vs. Trastuzumab) treated patients could be biased by the study design with stricter selection of patients, sequential instead of concurrent use of anthracyclines, closer monitoring of cardiac function during therapy, and cessation of therapy when cardiac dysfunction is detected. This idea is supported by studies in cardiomyocytes showing that concurrent use of Lapatinib and doxorubicin induced greater cardiomyocyte damage [94].

Pertuzumab: An analysis of cardiac safety data was undertaken by Lenihan *et al.* in 2012. A complete database of patient treated with Pertuzumab was used to review the incidence of asymptomatic LVEF dysfunction and symptomatic heart failure in 598 patients. Of those patients, 331 were treated with Pertuzumab alone, 175 in combination with a non-anthrocycline containing cytotoxic agent and 93 in combination with Trastuzumab. In the Pertuzumab as a single agent, 23 patients (6.9%) developed asymptomatic LVEF dysfunction and 1 patient (0.3%) developed symptomatic heart failure. In the combination with a non-anthrocycline containing cytotoxic group, 6 patients (3.4%) developed asymptomatic LVEF dysfunction and 2 patients (1.1%) developed symptomatic heart failure. In the combination with Trastuzumab group, 6 patients (6.5%) developed asymptomatic LVEF dysfunction and 1 patient (1.1%) developed symptomatic heart failure [95].

T-DM1, Afatinib and Naratinib: In initial phase I-II and recent EMILIA, a phase III clinical studies show that T-DM1 has a better cardiac safety profile compared to Trastuzumab. Only 1.7% of patients that treated with T-DM1 experienced decreased LVEF more than 15%. The cardiac safety of Afatinib and Naratinib is still being evaluated in clinical trials [96].

MECHANISMS OF HER2 INHIBITION INDUCED CARDIOTOXICITY

The HER2 receptor is pivotal for maintaining the physiological function of the adult heart, especially in the presence of cardiac stress, such as doxorubicin [10, 13, 97]. Although HER2 has no-known ligands, it is "primed" to form heterodimers with other HER receptors to propagate potent survival and growth signals [34]. The signaling pathways downstream of HER receptors, including the PI3K and MAPK pathways, govern key functions of the cell, including cardiomyocytes [33]. Understanding how HER2 protects the heart is, therefore, crucial for developing new therapeutic measures to prevent or reduce the cardiotoxicity caused by anti-HER2 cancer therapy. In this regard, it is especially important to understand the role of HER2 in the setting of the doxorubicin-injured heart.

The cardiac protective role of HER2 has been studied in transgenic mouse models with cardiomyocyte-specific conditional knockout of the HER2 gene as well as isolated cardiomyocyte treated with inhibitors [11, 97-99]. Studies have suggested that HER2 protects the heart by several mechanisms which include maintaining mitochondrial integrity, inhibiting cardiomyocyte apoptosis and oxidative stress, maintaining myofibril structure, and mediating the cardioprotective effects of NRG1 [11, 14, 16, 98-101].

HER2 inhibition on mitochondrial integrity and apoptosis

Studies in both mice and cultured cardiomyocytes suggest that HER2 inhibition may reduce mitochondrial integrity and increase pro-apoptotic signals [14, 98]. Mice with cardiomyocyte-conditional knockout of the HER2 gene (HER2-CKO) develop dilated cardiomyopathy around the age of 3 months [97]. Transmission electron microscopy reveals that the number of mitochondria and vacuoles in HER2-CKO ventricular myocardium is significantly increased, the phenotypes of which are also observed in human patients with anthracycline-induced cardiotoxicity. Mitochondrial function, however, is comparable to controls. Ligand-mediated PCR DNA fragmentation assay suggest that apoptosis is increased in HER2-CKO myocardium. Overexpression of the anti-apoptotic gene Bcl-xL in HER2-CKO mice partially rescues dilated cardiomyopathy [97].

In cultured rat neonatal cardiomyocytes, anti-HER2 antibody treatment increases ROS production, induces a loss of mitochondrial membrane potential and a decrease of ATP levels. These are associated with increased Bcl-xS/Bcl-xL ratio, translocation of BAX to mitochondria, increased cytochrome c release and caspase activation [98, 102]. In addition to the alteration of Bcl-2 family proteins, anti-HER2 antibody increases p53 proteins, which is associated with decreased activation of MDM2 (Mouse double minute 2 homolog), an E3 ubiquitin ligase that targets p53 for proteasome degradation [103]. Anti-HER2 antibody, however, does not further aggravate doxorubicin-induced apoptosis in neonatal cardiomyocytes [14]. In addition, in HER1/2 inhibitor treated adult rat cardiomyocytes, apoptosis was not detected [104]. Despite somewhat conflicting findings, these studies suggest that HER2 inhibition could cause mitochondrial damage and cardiomyocyte apoptosis. The discrepancy may be caused by different cell types and anti-HER2 antibody vs. inhibitors. Studies are needed to further investigate this mechanism in human cardiac tissues.

HER2 inhibition causes myofibril damage

In cultured adult rat cardiomyocytes, an anti-HER2 antibody or a HER1/HER2 inhibitor induces myofibril disarray and loss. In addition, HER2 inhibition and chemotherapy drugs (doxorubicin and paclitaxel) have additive effects on myofibril structure damage. In anti-HER2 antibody treated cardiomyocytes, myofibril damage is associated with decreased activation of ERK1/2 but not Akt. Inhibition of ERK1/2 by a kinase inhibitor induces a similar phenotype, suggesting the HER2 receptor may protect the myofibril structure by activating ERK1/2 [16, 105, 106].

HER2 inhibition changes the expression of genes that regulate key functions of the cell

EIZarrad *et al*. studied the gene expression profile in the heart from mice that are treated with Trastuzumab. They found that 243 transcripts are significantly changed by Trastuzumab treatment. The genes that are down-reglated by Trastuzumab include cytoskeletal proteins, sarcoplasmic reticulum transmembrane proteins, G-protein coupled receptors, growth factors, and ubiquitin ligase. These results suggest that Trastuzumab could damage cardiomyocytes by imparing cardiomyocyte contractile function, blocking survival signals, as well as DNA repair [107].

HER2 inhibition reduces the cardioprotective effects of Neuregulin1

The clinical findings in patients treated with Trastuzumab led to the discovery of the therapeutic role of Neuregulin1, a HER ligand, for heart failure [108, 109]. Preclinical studies show that NRG1s protect the heart from various stresses, such as doxorubicin, hypertension, ischemia, and viral infection [10, 11]. Recent clinical trials in heart failure patients show that injections of a recombinant NRG1 improve cardiac function [109]. The detailed biological functions and cardioprotective effects of NRG1 have previously been reviewed by us and others [7, 110-113]. Here we will focus on how HER2 inhibition may affect the cardioprotective effects of NRG1.

NRG1 proteins belong to the epidermal growth factor family [33]. NRG1s bind directly with HER3 and HER4, while recruiting HER2 as a co-receptor [33]. NRG1 proteins are synthesized and secreted by the endocardium and the endothelium of the cardiac microvasculature [113, 114]. In heart failure patients, the cardiac and serum NRG1s are increased [115-117], suggesting this could be a compensatory protective mechanism. Studies have shown that NRG1s protect the heart by multiple mechanisms which include maintaining myofibril structure, improving survival, growth and proliferation of cardiomyocytes, counterbalancing β-adrenergic effects and maintaining calcium homeostasis, improving angiogenesis and promoting stem cell differentiation into cardiomyocytes [11, 13, 14, 16, 101, 118-124]. Among these effects, the HER2 receptor is necessary for NRG1 to reduce apoptosis and oxidative stress, maintain cardiac troponins and the myofibril structure, as well as promote stem cell differentiation into the cardiac lineage [11, 12, 14, 17]. On other hand, activation of Akt and ERK1/2 by NRG1s is HER2-independent [14, 100].

Taken together, the HER2 receptor is crucial for maintaining multiple aspects of cardiac functions, cardiomyocyte survival and mediating cardioprotective signals of NRG1s.

CONCLUSION

Targeted cancer therapies aiming at inhibiting HER2 receptor tyrosine kinase signaling have significantly impoved cancer therapeutic effects and survival in breast cancer patients. The HER2 receptor and HER receptor ligands, including NRG1 proteins, however, are pivotal for protecting the heart from cardiac stress. Cardiac side effects are more severe in patients treated with certain anti-HER2 agents but less evident with others. Mechanisms behind these phenomena started being revealed. New cancer therapies targeting multiple HER receptors and HER ligands may inevitably cause more severe cardiotoxicity than those caused by single agents. The current strategies for managing cardiotoxicity include screening for pre-existing cardiovascular problems, sequential use of chemotherapy and anti-HER2 agents, closely monitoring patients during cancer therapy, and cessation of therapy when cardiac dysfunction is detected. These strategies have significantly reduced symptomatic cardiac dysfunction in anti-HER2 drug treated patients. However, a significant number of patients could be excluded from the most effective cancer therapy they need. The American Cancer Society reports that the five-year survival of all cancers diagnosed between 2003 and 2009 is 68%. The cardiac side effects caused by cancer therapy could become an additional health burden for cancer survivors or the cause of death. Preventative and therapeutic strategies are needed to overcome this emerging clinical challenge.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work is supported by the American Heart Association Grant-In-Aid (Yan X, 10GRNT4710003) and National Heart Lung and Blood Institute (Yan X, HL106098).

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Received: September 29, 2013 **Accepted: November 21, 2013**