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The Vulnerability of the Heart As a Pluricellular Paracrine Organ: Lessons From Unexpected Triggers of Heart Failure in Targeted ErbB2 Anticancer Therapy

Cardiovascular Disease: Hidden Killer in Adult Survivors of Childhood Cancer

Emerging Anticancer Strategies: Is There Cause for Concern? Yes!

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The Vulnerability of the Heart As a Pluricellular Paracrine Organ

Lessons From Unexpected Triggers of Heart Failure in Targeted ErbB2 Anticancer Therapy

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Abstract: In this review, we address clinical aspects and mechanisms of ventricular dysfunction induced by anticancer drugs targeted to the ErbB2 receptor. ErbB2 antagonists prolong survival in cancer, but also interfere with homeostatic processes in the heart. ErbB2 is a coreceptor for ErbB4, which is activated by neuregulin-1. This epidermal growth factor–like growth factor is released from endothelial cells in the endocardium and in the myocardial microcirculation, hence contributing to intercellular crosstalk in the ventricle. We look at the physiological aspects of neuregulin-1/ErbB signaling in the ventricle, and review its (mal)adaptive responses in chronic heart failure. We also compare structural aspects of ErbB receptor activation in cancer and cardiac cells, and analyze the mode of action of current ErbB2 antagonists. This allows us to predict how these drugs interfere with paracrine processes in the ventricle. Differences in the mode of action of individual ErbB2 antagonists affect their impact on the function of the ventricle, considered to be “on-target” or “off-target.” Establishing the relation between the cardiac side effects of ErbB2 antagonists and their impact on paracrine ventricular control mechanisms may direct the design of a next generation of ErbB2 inhibitors. For cardiologists, there are lessons to be learned from the unexpected side effects of ErbB2-targeted cancer therapy. The vulnerability of the heart as a pluricellular paracrine system appears greater than anticipated and intercellular crosstalk an essential component of its functional and structural integrity. (*Circ Res.* 2010;106:35-46.)

Key Words: heart failure ■ ErbB receptors ■ trastuzumab ■ neuregulin-1

The ErbB family of receptor tyrosine kinases and their ligands are important in the maintenance of structural and functional integrity of the adult heart. Activation of ErbB receptors in the heart is controlled by the ErbB4 ligand neuregulin (NRG)-1. This epidermal growth factor (EGF)-

like growth factor is released from endothelial cells in the endocardium and microcirculation of the myocardium. NRG-1 induces a “cardioprotective program” in vitro and improves cardiac function in models of heart failure in vivo. In cancer, gene amplification and overexpression of ErbB2

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Non-standard Abbreviations and Acronyms

ADAM	a disintegrin and metalloproteinase
EGF	epidermal growth factor
ERK	extracellular signal-regulated kinase
GPCR	G protein-coupled receptor
HB-EGF	heparin-binding epidermal growth factor–like growth factor
NRG	neuregulin
PI3K	phosphoinositide 3-kinase

leads to ligand-independent ErbB2-ErbB3 heterodimer complexes and to uncontrolled tumor growth. Targeted anticancer therapy against ErbB2 with trastuzumab (Herceptin) greatly improves cancer prognosis. It also induces ventricular dysfunction, however, most significant when given in combination with anthracyclines. This side effect has limited the use of trastuzumab in a subset of patients.

Unraveling the mechanisms of trastuzumab-induced cardiomyopathy has been more difficult than anticipated. Despite growing insights in the structural physiology of ErbB receptors and in the mode of action of trastuzumab, it is still unclear how precisely trastuzumab-induced cardiomyopathy culminates from so-called “on-target” toxicity by impairment of NRG-1–induced ErbB signaling. Other ErbB2-dependent, as well as ErbB2-independent, mechanisms may be involved. New ErbB2 antagonists, like lapatinib and pertuzumab, seem to affect ligand-induced ErbB signaling in a more direct fashion than trastuzumab. The cardiac side effects of the latter drugs are only beginning to emerge. These data will be crucial to establish the cardiotoxic mechanisms of ErbB2-antagonists. They could also influence the design of future anticancer therapies in an attempt to retain anticancer effects, while minimizing cardiac toxicity.

We will review the biology of ErbB signaling in cancer and cardiac cells, the regulatory aspects of the NRG-1/ErbB system in heart failure, and the emerging opportunities to target this system with drugs. We also analyze the working mechanisms of different ErbB2-targeted cancer drugs and the reasons why these drugs may induce ventricular dysfunction and heart failure. We will conclude with reflections on how unexpected triggers of heart failure in cancer therapy influence our concepts that describe the function of the heart in health and disease.

Targeted Anti-ErbB2 Therapy in Cancer and Its Unexpected Side Effect

ErbB2 in Cancer

Cancer therapy has made a tremendous progress with the development of targeted therapies. These therapies, usually directed against tyrosine kinases, halt cancer cell proliferation and metastasis without nonspecific cytotoxicity to other rapidly dividing cells. Targeted therapies are usually combined with traditional regimens, and have been proven to significantly reduce cancer progression and mortality.

One of the first therapeutics in this (r)evolution is trastuzumab (Herceptin), a recombinant humanized monoclonal

antibody to ErbB2, also known as HER-2 (human epidermal growth factor receptor-2) (from which the name Herceptin is derived). ErbB2 is a receptor tyrosine kinase known to be overexpressed in approximately 25% of all breast tumors and is associated with a poor prognosis and decreased overall survival.¹ ErbB2 belongs to the family of human epidermal growth factor (EGF) receptors consisting of the EGF receptor (ErbB1), ErbB2, ErbB3, and ErbB4.² This family of growth receptors is responsible for the regulation of cellular metabolism, growth and survival. ErbB2 has no known ligand, instead, it has a fixed conformation that resembles the ligand-activated state.³ This structural characteristic is the reason why uncontrolled gene amplification and ErbB2 overexpression leads to ligand-independent hetero-dimerization of ErbB2, continuous stimulation of downstream signaling pathways and uncontrolled cellular proliferation (Figure 1). Recent studies have established that the ErbB2/ErbB3/phosphoinositide 3-kinase (PI3K) complex forms the major oncogenic unit in ErbB2 amplified tumor cells, and that subsequent activation of Akt is the major oncogenic mechanism.⁴ The antiproliferative activity of trastuzumab is directly linked to its ability to disrupt the ErbB2-ErbB3 complex in tumor cells, reducing the activity of intracellular Akt. Other previously proposed antiproliferative mechanisms of trastuzumab in ErbB2-positive cancer cells, including downregulation of ErbB2 abundance^{5,6} and ErbB2 dephosphorylation,⁷ seem now less likely.^{8,9}

In summary, our understanding of ErbB signaling and the related working mechanisms of trastuzumab in cancer cells has expanded in the past years. This information will help us to unravel the effects of ErbB2 antagonists on ventricular function. Some clinical aspects of this topic are introduced in the next paragraph.

Trastuzumab and Its Unexpected Effects on Ventricular Function

Trastuzumab was first approved by the Food and Drug Administration in 1998 for the treatment of advanced metastatic breast carcinoma with overexpression of ErbB2.¹⁰ Since 2006 its use is broadened to the treatment of early stage breast cancer. Addition of trastuzumab to standard regimens reduces breast cancer relapse by 50% and mortality by 33%.^{11–13} Following these astonishing results trastuzumab was tested in broader oncological indications and novel ErbB2 inhibitors were developed.¹⁴ Unfortunately, a major down-side to the “trastuzumab success story” emerged: its unexpected effects on ventricular function.

The cardiac side effect of trastuzumab was first noticed following the pivotal trials leading to its approval by the Food and Drug Administration in 1998.¹⁵ In these trials, trastuzumab was administered on top of standard therapy, which consisted of either paclitaxel or doxorubicin and cyclophosphamide. Post hoc analyses revealed up to 11% “cardiotoxicity” in patients receiving trastuzumab on top of paclitaxel compared with only 1% to 4% in those who received paclitaxel alone. There was an even greater incidence of cardiotoxicity in patients receiving the combination of trastuzumab and anthracyclines. Anthracyclines, such as doxorubicin, are themselves cardiotoxic,¹⁶ but addition of trastu-

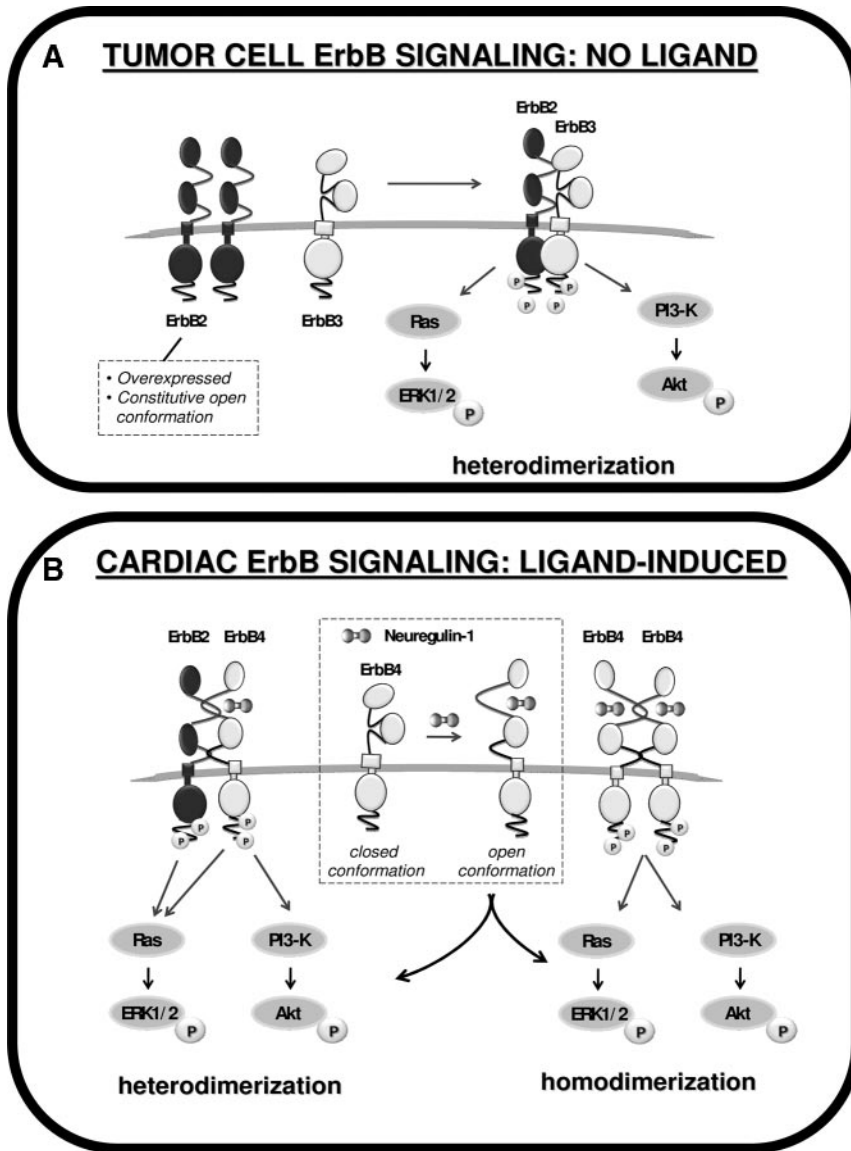


Figure 1. ErbB signaling in ErbB2-overexpressing tumor cells and in cardiomyocytes. **A,** Ligand-independent ErbB signaling in ErbB2-overexpressing cells. The high abundance of ErbB2 with its constitutive open conformation recruits other ligand-unbound ErbB family members for dimerization. Recent studies have shown that the ErbB2-ErbB3-PI3K-p85 complex forms the major oncogenic unit, and that trastuzumab disrupts this complex. **B,** Ligand-dependent ErbB signaling in cardiomyocytes. In cardiomyocytes expressing low levels of ErbB2, NRG-1 binding to ErbB4 induces a switch from the closed to the open conformation, exposing the domain II dimerization arm. This ligand-induced event leads to the formation of ErbB4/ErbB4 homodimers or ErbB2/ErbB4 heterodimers. Dimerization partners induce mutual transphosphorylation and activation of docking sites for signaling molecules. This leads to activation of downstream signaling cascades. In adult cardiomyocytes, missing ErbB3, only ErbB4 connects to the PI3K-Akt pathway.

zumab leads to a synergistic increase in incidence of cardiac symptoms from 13% (with doxorubicin alone) to 27% (when combined with trastuzumab). Moreover, severe chronic heart failure (New York Heart Association class III and IV) occurred in 16% of patients treated with this combination.

Following these observations, well-designed prospective cardiac monitoring was included as a safety measure in all new trastuzumab trials. Also, treatment regimens were altered, initiating treatment with trastuzumab only after completion of anthracycline therapy.¹⁷ Although these measures reduced the incidence of symptomatic chronic heart failure to less than 5%, asymptomatic cardiac dysfunction still occurs in 14% of patients receiving the trastuzumab+anthracycline combination and in 7% receiving trastuzumab alone.¹⁸⁻²⁰ Trastuzumab-induced ventricular dysfunction remains a relevant problem and is the leading cause of treatment withdrawal.

In summary, the unexpected finding of trastuzumab-induced ventricular dysfunction has sparked great scientific efforts into the elucidation of the underlying mechanisms. From its original discovery, it was noticed that effects of

trastuzumab on ventricular function were dependent on the clinical circumstances of administration, especially with relation to its temporal relation with anthracyclines. It was observed that the effects were dose-independent and largely reversible within 1 to 3 months. This has led to the general paradigm that targeting the ErbB2 receptor is not cardiotoxic per se but, instead, that it could block some homeostatic pathways in the heart. According to the hypothesis, which is still valid, ErbB2-dependent pathways are indispensable to withstand, or to recover from, cardiac injury imposed by stress factors.^{21,22} As a result, the scientific focus shifted to the as yet undefined role of the ErbB2 and other ErbB receptors in cardiac physiology.

Direct Molecular, Cellular, and Tissue Responses of ErbB Activation in the Heart

ErbB signaling in the heart was known to be indispensable for normal fetal cardiac development before the discovery of trastuzumab-induced ventricular dysfunction. Studies in mutant mice revealed that gene deletion of ErbB2 and ErbB4 or

their ligand NRG-1 leads to embryonic lethality caused by abnormal ventricular trabeculation.^{23–25} ErbB3-null mice display a different phenotype and die because of defective cardiac cushion formation.²⁶ Of note, deletion of ErbB1 leads to embryonic or early postnatal lethality which appears not to be primarily of cardiac origin.^{27,28} Cardiac expression of ErbB ligands and receptors was shown to persist into adulthood, suggesting a role in postnatal cardiac physiology.²⁹

ErbB1, ErbB2 and ErbB4 appear to be the principal ErbB receptors in the postnatal heart. ErbB3 is not detectable in adult cardiomyocytes.²⁹ In nontumor cells, activation of the ErbB receptors is controlled by spatial and temporal expression of their ligands. Principal ligands in the heart are EGF (binding to ErbB1), NRG-1 (binding to ErbB4), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) (binding to ErbB1 and ErbB4), although a physiological role for other ligands like epiregulin and amphiregulin are not excluded.

NRG-1 Signaling Through ErbB4 and ErbB2 in the Heart

Molecular Responses

NRG-1 is synthesized as a transmembrane protein in myocardial microvascular and endocardial endothelial cells,^{30,31} and cleaved into the interstitial space by metalloproteinases from the ADAM (a disintegrin and metalloproteinase) family.^{32,33} NRG-1 binds to ErbB4 receptors on cardiomyocytes. This leads to a conformational change of ErbB4 from a closed to an open state, exposing the subdomain II dimerization arm, which then leads to formation of ErbB4-ErbB4 homodimers or ErbB4-ErbB2 heterodimers (Figure 1).³⁴ Transphosphorylation of the ErbB dimer members creates binding sites for signaling proteins harboring Src homology 2 or phosphotyrosine binding motifs.³⁵ Following this sequence of events, NRG-1 activates PI3K/Akt, extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein kinase pathways, and the focal adhesion kinase.³⁶

Recently, proteomic studies searching for the phosphotyrosine interactome of the ErbB receptor family have identified that the different family members differ in their preferred interaction partners (Figure 2). This indicates distinct roles in signaling.^{37,38} Interestingly, only ErbB3 and ErbB4, but not ErbB2 and ErbB1, display docking sites for the PI3K subunit p85.^{35,37} This means that in adult cardiomyocytes, which do not express ErbB3, ErbB-dependent activation of Akt relies on activation of ErbB4. This also means that, as long as NRG-1-induced activation of ErbB4 is preserved, and subsequent signaling through ErbB4-ErbB4 homodimers occurs, NRG-1 activates Akt in cardiomyocytes. This scenario may explain why ErbB2 antagonists like lapatinib do not affect NRG-1-mediated Akt activation in cardiomyocytes (K.D. and G.W.D.K., unpublished observation, 2009) but exert antiproliferative effects through inhibition of Akt in ErbB2 amplified tumor cells. Indeed, as explained above, activation of Akt in ErbB2-amplified tumor cells is mediated by the oncogenic complex ErbB2/ErbB3/PI3K, which is sensitive to lapatinib.

In summary, ultrastructural and proteomic analyses of ErbB receptors have formed a firm molecular basis to study the physiology of NRG-1/ErbB signaling and to explain the

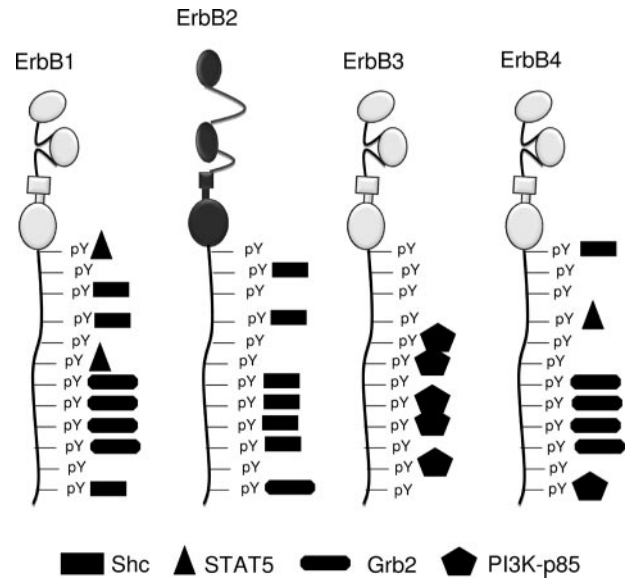


Figure 2. Schematic presentation of phosphotyrosine interaction partners of ErbB family members. Schematic (and incomplete) presentation of ErbB interaction partners, as identified by proteomic studies described elsewhere.³⁶ The interaction partners are indicated by symbols. The figure shows that each ErbB family member has characteristic preferences for interacting proteins. ErbB1 has multiple interaction partners and several binding sites for Grb2. ErbB2 has few interaction partners; of them Shc is the most common. ErbB3 interacts mainly with PI3K subunit p85. ErbB3 is, however, absent in the adult heart. ErbB4 shows a diversity of interaction partners. In absence of ErbB3 in the adult heart, activation of ErbB4 is the only way to the activation of the PI3K-Akt pathway by ErbB ligands.

impact of ErbB2 antagonists on ErbB signaling in the ventricle.

Cell and Tissue Responses

Figure 3 summarizes the direct responses of cardiac tissue to NRG-1. Most of these observations have been made in vitro. Together with phenotypic observations of gene deletion studies in mice, these responses have led to the general consensus that NRG-1 promotes a “cardioprotective” program. Antiapoptotic pathways,^{29,39,31} hypertrophic^{29,31} and even mitotic⁴⁰ growth, cell elongation with improved cell-cell adhesion,³⁶ angiogenesis,⁴¹ and reduced sensitivity to adrenergic stimulation⁴² may all contribute to NRG-1-mediated cardioprotection. Whether NRG-1 is truly cardioprotective in heart failure through attenuation of adrenergic stimulation needs to be confirmed, especially because increasing adrenergic stimulation in these conditions has cardioprotective actions.⁴³ The phenotype of the heterozygous NRG-1 mice, showing greater sensitivity to anthracyclines in terms of survival, reduction of body mass, and cardiac performance, has reinforced in vitro observations on the antiapoptotic effects of NRG-1.⁴⁴ By contrast, ventricular apoptotic cell death in cardiac-specific ErbB2 knockouts has been shown to be minor or absent,⁴⁵ detectable only with very sensitive assays, in turn calling into question the relevance of some in vitro observations. In fact, despite the identification of several “protective” responses in vitro, cardioprotective mechanisms induced by NRG-1 in vivo remain largely unexplained. Mouse models with conditional, single gene deletions of

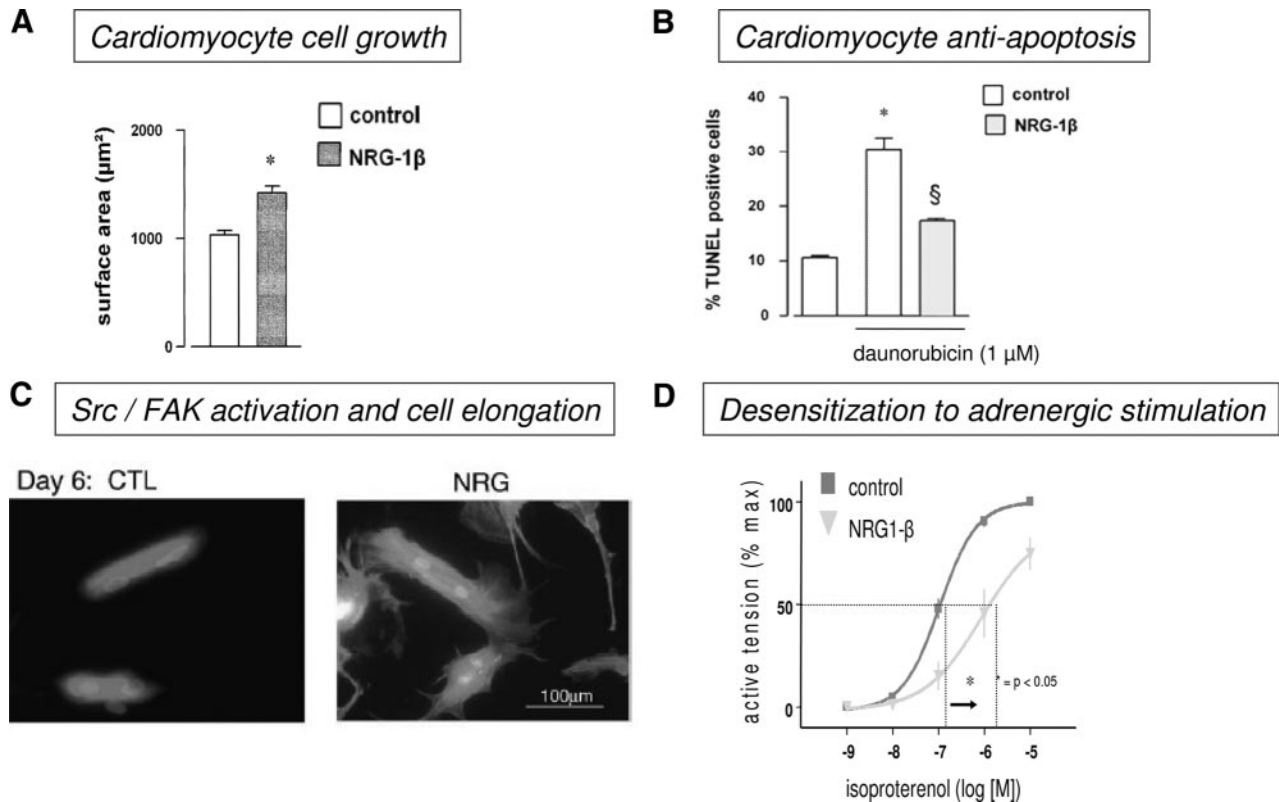


Figure 3. Responses of cardiomyocytes to NRG-1. After the observation that gene deletion of NRG-1 or of ErbB receptors results in cardiac malformation or dilated cardiomyopathy, cardiac cell and tissue responses to NRG-1 have been studied. NRG-1 was observed to induce cardiomyocyte hypertrophy (modified from Lemmens et al³¹) (A) and to increase cardiomyocyte resistance to apoptotic cell death (modified from Lemmens et al³¹) (B). C, In addition, NRG-1 induced cell extensions of lamellipodia that resulted in restoration of cell-to-cell contact between isolated myocytes, allowing for synchronous beating in vitro (reprinted from Kuramochi et al³⁶ with permission). Also, NRG-1 administered to isolated, twitching papillary muscles induced a nitric oxide synthase-dependent desensitization of cardiac muscle to the inotropic effects induced by β_1 -adrenergic agonists (modified from Lemmens et al⁴²) (D).

ErbB receptors, showing a dilated cardiomyopathy phenotype,^{45,46} have helped to establish the physiological importance of ErbB receptors in the postnatal heart but have added little information to the underlying mechanisms of cardioprotection induced by NRG-1. Liu et al observed that chronic daily intravenous administration of NRG-1 improves ventricular function and hemodynamics and reduces neurohormonal activation and mortality in rodent models of ischemic, inflammatory and toxic cardiomyopathy.⁴⁷ Although adding little mechanistic information, this study reinforced the importance of further studying the direct effects of NRG-1 on cardiac function in vivo. Recently, Bersell et al showed that injecting NRG-1 in adult mice induced cardiomyocyte cell cycle activity and promoted myocardial regeneration, leading to improved function after myocardial infarction.⁴⁰ Undifferentiated progenitor cells did not contribute to NRG-1-induced cardiomyocyte proliferation. This study may be a breakthrough linking observations of NRG-1-induced cardiomyocyte protection in vitro to the preservation and recovery of ventricular function in vivo. Whether the recovery from trastuzumab-induced cardiomyopathy within a time-frame of 1 to 3 months can be explained by NRG-1-induced cardiomyocyte cell division is currently unknown and should be studied.

One attractive alternative mechanism of cardioprotection by NRG-1/ErbB signaling may be the activation of cardio-

myocyte NO synthase⁴² and the subsequent increase in myocardial cGMP content. Although this mechanism is still relatively unexplored, NRG-1 may be one of the modulators that increase myocardial cGMP, acting in a cooperative fashion with NO released by surrounding cardiac endothelial cells, and with natriuretic peptides. Endogenous production of cGMP in cardiomyocytes, and thus activity of the NRG-1/ErbB signaling system, may also predict the cardioprotective efficacy of phosphodiesterase-5 inhibitors. Indeed, as recently discussed by Kass et al, cardiac diseases with insufficient cGMP synthesis may not respond to phosphodiesterase-5 inhibition.⁴⁸ Hence, combined therapy with recombinant NRG-1 and phosphodiesterase-5 inhibitors may be an attractive approach for cardioprotection and reverse remodeling.

In summary, gene deletion studies in mice have firmly established an indispensable role of ErbB signaling in the regulation of cardiac function in vivo. In vitro studies have emphasized the effects of the ErbB4 ligand NRG-1 and have provided clues toward the underlying cellular and molecular mechanisms of protective ErbB signaling in cardiomyocytes. Animal studies are now beginning to translate these NRG-1-mediated mechanisms back into physiological processes regulating cardiac function in vivo. Given the endothelial origin of NRG-1 in the ventricle, these studies underscore the importance of intercellular crosstalk in the heart. The pluri-

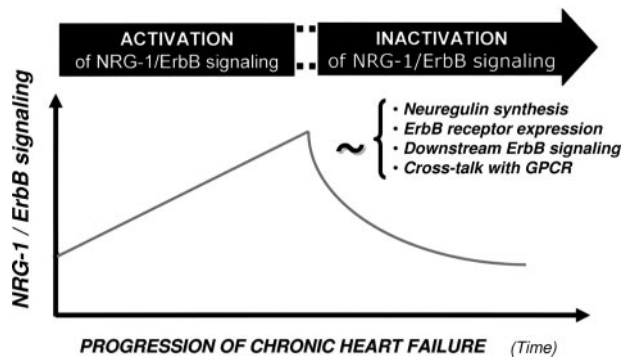


Figure 4. Biphasic course of NRG-1/ErbB signaling in heart failure. During progression of chronic heart failure, activity of the NRG-1/ErbB system evolves in 2 phases. After an initial phase of activation, activity of the system decreases. Given the cardioprotective actions of this system, activation is considered to be adaptive and protective, whereas inactivation may contribute to the progression of the disease. The 4 levels of variability of this system are indicated and further discussed in the text and in Figure 5. These levels are important because they may define targets for medical therapy.

cellularity and the related paracrine mechanisms of homeostasis are indispensable for the heart's functional and anatomic integrity.

ErbB1 Signaling in the Heart

Apart from being a dimerization partner for ErbB4, ErbB2 also dimerizes with ErbB1 after binding with ligand-bound ErbB1. EGF and HB-EGF seem to be the most important ligands of ErbB1 in the heart. EGF, HB-EGF and ErbB1 are expressed in the adult heart, but their role in the regulation of adult cardiac function remains incompletely understood. Of note, anticancer therapy targeted solely to ErbB1 (erlotinib, gefitinib) has no major cardiac side effects.⁴⁹ Based on current studies that we have read on this subject,^{50–57} we conclude that the cardiotoxic effects of most ErbB1-targeted interventions (including conditional expression of the ErbB1 gene) may be an indirect manifestation of impaired ErbB2 signaling.

(Mal)adaptive Changes of ErbB Signaling in Cardiac Disease

Growing evidence for cardioprotective actions of NRG-1 in the heart has led to the hypothesis that the cardiac NRG-1/ErbB system may be part of a compensatory system, activated to counterbalance maladaptive forces in heart failure.⁵⁸ Given the cardiac endothelial origin of NRG-1 in the heart, activation of this system in heart failure would be a manifestation of intensified endothelium-cardiomyocyte crosstalk.⁵⁹ Although some studies support the activation of the NRG-1/ErbB system in heart failure, other studies have shown that activity of the system is reduced. These contrasting observations are, most likely, explained by the biphasic course of NRG-1/ErbB activity during the progression of the disease (Figure 4). After an initial period of adaptive activation, that may protect the heart, NRG-1/ErbB signaling becomes maladaptively depressed, perhaps to release the NRG-1-induced “brake” on β -adrenergic signaling and thus to allow more inotropic support to the failing ventricle. Depression of NRG-1/ErbB signaling may ultimately constitute a detrimen-

tal event in the progression of the disease, especially if it would be paralleled by simultaneous downregulation of other protective pathways.

The fluctuating intensity of NRG-1/ErbB signaling in heart failure is generated by at least four (mal)adaptive changes in NRG-1/ErbB system. The importance of each of these changes may vary among different forms of heart failure. We briefly discuss each of these variables separately (Figure 5).

NRG-1 Synthesis by the Cardiac Endothelium

Experiments in rodents with transaortic constriction–induced cardiac overload have revealed that levels of ventricular NRG-1 progressively increase during development of concentric ventricular hypertrophy.³¹ During the transition to eccentric hypertrophy and the development of pump dysfunction, however, NRG-1 expression drops (Figure 5A). The underlying mechanisms of these changes remain incompletely explained. Interestingly, studies with isolated cardiac endothelial cells (the main source of NRG-1 in the heart) have indicated that mechanical strain increases endothelial NRG-1 synthesis and release. Angiotensin II and adrenergic agonists have opposite effects.³¹ These observations allow us to speculate that increased NRG-1 synthesis in the ventricle, evoked by local mechanical forces in early disease, become suppressed by activation of neurohormonal factors once pump failure develops. In addition, NRG-1 release from the endothelium is acutely promoted in models of ischemia/reperfusion.⁶⁰ In chronic heart failure of ischemic but not of nonischemic etiology, circulating NRG-1 levels are increased.⁶¹ This suggests that ischemia may also be an important trigger for endothelial NRG-1 synthesis and release.

Expression of ErbB2 and ErbB4 Receptors by Cardiomyocytes

Studies by Rohrbach and coworkers have established that ventricular expression of ErbB2 and ErbB4 receptors becomes markedly depressed in advanced heart failure.^{62,63} Similarly as for NRG-1, ErbB expression was preserved in the early stages of the disease (Figure 5B). The mechanisms of ErbB downregulation in advanced heart failure remain unknown, although mechanically unloading the ventricle with an assist-device, restored ErbB expression.⁶³

Reduced availability of ErbB receptors separates cardiomyocytes from protective influences by cardiac endothelial cells, ie, myocardial capillary and endocardial cells. We recently observed that in obese leptin-resistant mice with diabetes, in the absence of ventricular pump failure, baseline activity of ErbB2 and ErbB4 is markedly downregulated (K.D. and G.W.D.K., unpublished data, 2009). These observations extend the ErbB downregulation in advanced heart failure to conditions that increase the risk to develop heart failure. Accordingly, preventing or reversing this phenomenon may provide interesting targets for therapy.

Downstream Signaling by Ligand-Activated ErbB Receptors

Ligand binding and activation of ErbB receptors in the normal heart is rapidly followed by phosphorylation of the downstream effectors ERK1/2 and Akt. Surprisingly, in a

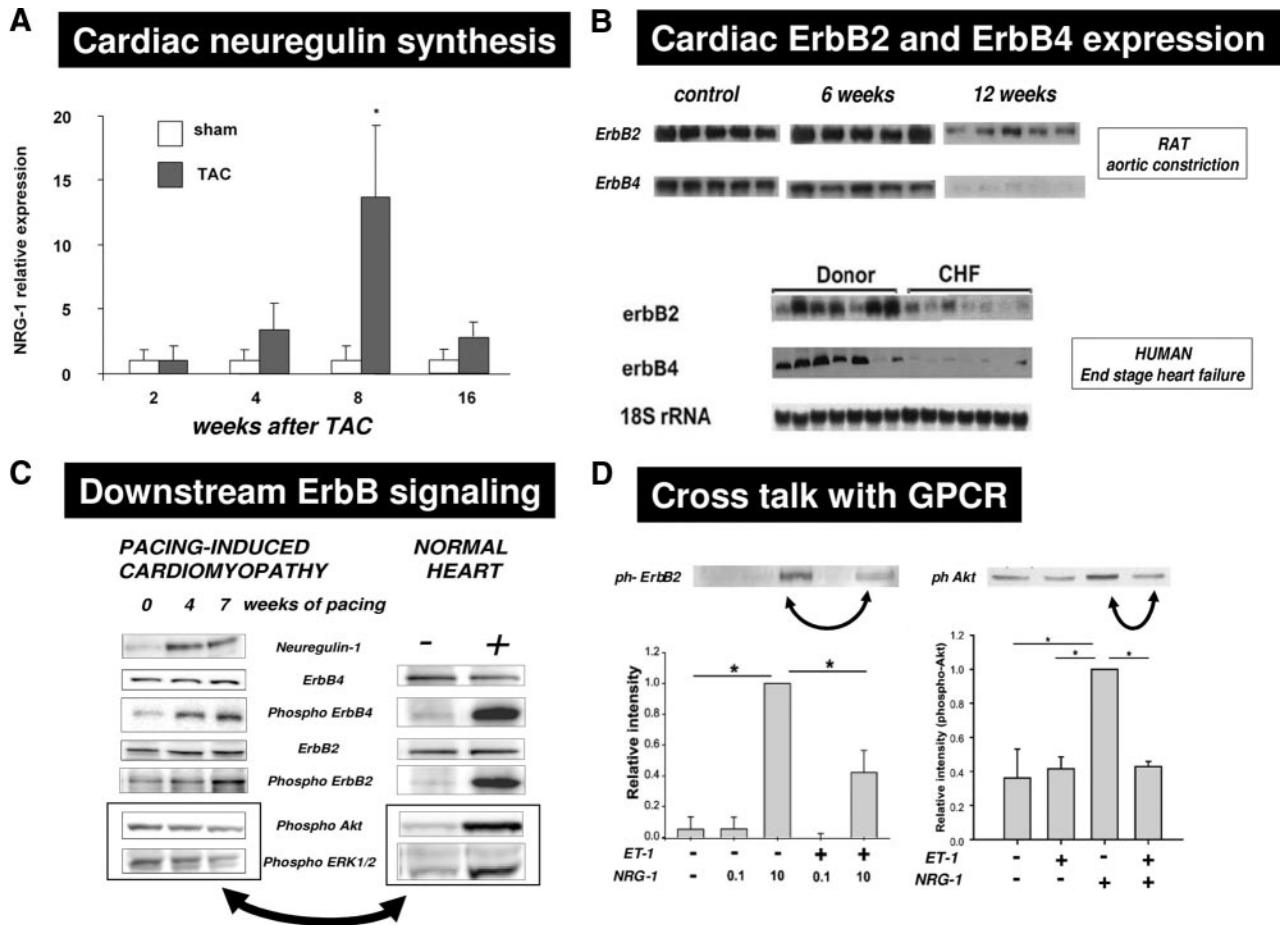


Figure 5. (Ma)adaptive changes of ErbB signaling in cardiac disease. NRG-1/ErbB signaling shows at least four levels of adaptation during progression of cardiac disease. A, Cardiac synthesis of NRG-1. Studies of transaortic constriction (TAC) in the rat showed that mRNA and protein levels of NRG-1 were increased during phases of concentric left ventricular hypertrophy (up to 8 weeks after initiation of transaortic constriction) but then normalized when eccentric hypertrophy developed (modified from Lemmens et al⁹¹). B, Cardiac ErbB2 and ErbB4 expression. Studies of transaortic constriction in the rat and in human end-stage heart failure have shown that expression of ErbB receptors robustly decreases at stages of advanced disease (modified from Rohrbach and colleagues^{62,63}). C, Downstream ErbB signaling. Study in pacing-induced heart failure revealed that during cardiomyopathy, downstream effectors of ErbB receptors ERK1/2 and Akt remained inactivated, despite clear evidence of activation of NRG-1 synthesis and ErbB receptor phosphorylation. Downstream signaling thus appeared to be abrogated (modified from Doggen et al⁶⁴). D, Inhibitory crosstalk with GPCRs. Study in isolated cardiomyocytes showing that activation of the endothelin-A receptor induced attenuation of NRG-1-induced ErbB receptor phosphorylation and of downstream Akt signaling (reprinted from Chung and Walker⁶⁸ with permission).

model of pacing-induced ventricular dilation and pump failure, at stages where NRG-1 expression and phosphorylation levels of ErbB2 and ErbB4 were increased, ERK1/2 and Akt remained paradoxically silent (Figure 5C).⁶⁴ These observations contrast with studies in the physiological setting of pregnancy-induced ventricular hypertrophy in which activation of NRG-1, ErbB2/4, ERK1/2 and Akt coincided (K.L. and G.W.D.K., unpublished observation, 2009). One possible explanation is that inhibitory pathways, activated during “pathophysiological” but not “physiological” hypertrophy would also act at the post-ErbB receptor level, interfering with the activation of downstream ErbB receptor effectors. To what extent these pathways attenuate, change or irreversibly abrogate the cardioprotective ErbB program deserves further investigation.

Crosstalk Between ErbB Receptors and G Protein-Coupled Receptors

Both in cardiomyocytes and other noncardiac cells types, G protein-coupled receptor (GPCR) agonists (angiotensin II,

endothelin-1, and β_1 -adrenergic agonists) transactivate ErbB receptors.^{54,65,66} More specifically, activation of GPCRs has been shown to induce tyrosine phosphorylation of ErbB1 and ErbB2.⁶⁶ Effects on ErbB4 are less well established. In cardiomyocytes, cross-communication between these signaling systems has been involved in the establishment of prohypertrophic and cell viability regulating effects of GPCR stimulation.⁶⁵ As reviewed by Fuller et al, however, there are some inconsistencies in this field that challenge the physiological meaning and the proposed mechanisms of GPCR-induced activation of ErbB signaling.⁶⁷

To complicate this issue even further, recent observations have suggested that GPCR stimulation may actually attenuate ligand-induced ErbB signaling, especially with regard to NRG-1/ErbB4-induced signaling. In a study in isolated cardiomyocytes by Chung et al, in which ErbB4 was identified as a binding partner of the endothelin-A receptor, endothelin-1 inhibited NRG-1-induced activation of ErbB4, ErbB2, and the downstream effectors Akt and ERK1/2 (Figure 5D).⁶⁸ In our labora-

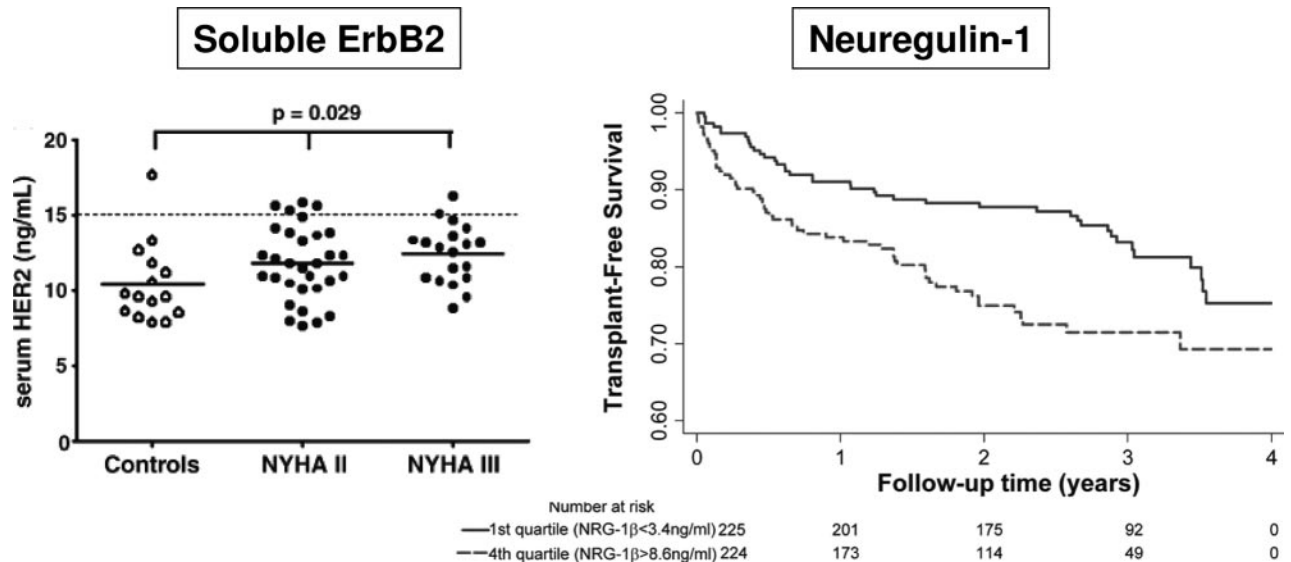


Figure 6. Serum biomarkers of NRG-1/ErbB activity in heart failure. Left, Serum levels of the shed extracellular domain of ErbB2 (HER-2) in outpatients with chronic heart failure, according to the symptoms following the New York Heart Association classification (reprinted from Perik et al⁶⁹ with permission). Right, Transplant-free survival in outpatients with chronic heart failure according to quartiles of serum levels of NRG-1 β (first versus fourth quartile (reprinted from Ky et al⁶¹ with permission)).

tory, we observed similar effects with angiotensin II which dose-dependently attenuated ErbB4 and ErbB2 activation through activation of the angiotensin type 1 receptor (K.D. and G.W.D.K., unpublished observations, 2009).

In summary, interaction between GPCRs and ErbB receptors is complex. Besides transactivating ErbB1/ErbB2 signaling, there is emerging evidence that GPCRs may inhibit ligand-induced ErbB4 signaling by NRG-1 (“transinhibition”). It may be somewhat premature to speculate on the physiological meaning of these observations. We personally believe that further study of this issue may disclose crucial information on the regulation of ErbB signaling in cardiac disease. GPCRs may be among the decisive players that can switch intracellular signaling components of ErbB receptors from signaling to nonsignaling, or vice versa. If confirmed, these events have a major pathophysiological significance and may carry important therapeutic potential.

To summarize this section, current studies show that the NRG-1/ErbB signaling system is transiently activated during the progression of heart failure. The transition from activation of the NRG-1/ErbB system toward inhibition coincides with the development of ventricular pump dysfunction and may be caused by reduced ligand synthesis, by reduced ErbB receptor expression, by abrogated downstream ErbB receptor signaling, and by crosstalk with GPCR signaling. Serum biomarkers that reflect the activity of cardiac ErbB-signaling may be meaningful. A recent study showed that serum levels of NRG-1 β , measured in outpatients with heart failure and reduced left ventricular ejection fraction, were significantly elevated in patients with worse disease severity. NRG-1 β serum level was an independent risk factor for death over a median follow-up of 2.4 years, most pronounced in patients with ischemic cardiomyopathy (Figure 6).⁶¹ In another study, the serum levels of the soluble fraction of ErbB2, shed from the membrane after activation, was slightly but significantly increased in outpatients with chronic heart failure, and

correlated with left ventricular ejection fraction and the symptoms of the patient.⁶⁹ In a larger study, enrolling patients undergoing coronary angiography, serum ErbB2 levels, however, were not influenced by left ventricular ejection fraction or by left ventricular filling pressures.⁷⁰

Mechanisms of Cardiotoxicity of Targeted ErbB Therapy: What Is the Relation With NRG-1/ErbB Signaling?

In the previous paragraphs, we have reviewed the clinical problem of cardiotoxicity induced by anti-ErbB2 treatment with trastuzumab in cancer and discussed the (patho)physiology of the ErbB receptor system in the heart. Mostly based on the analogy between ErbB2 knockout-induced cardiomyopathy and trastuzumab-induced heart failure, many have concluded that trastuzumab causes heart failure by blocking the physiological actions of ErbB2 in the heart. Although logical and attractive, this hypothesis remains unproven. In fact, recent observations, mostly on the ultrastructural actions of trastuzumab on the ErbB2 receptor, have challenged this hypothesis. Let us explain.

In tumor cells, ErbB signaling is triggered by gene amplification and overexpression of ErbB2 receptors, leading to ligand-independent “constitutive” ErbB2-ErbB3 heterodimer formation.⁴ Trastuzumab binds to subdomain IV of ErbB2 and disrupts these constitutive heterodimers, although the exact mechanisms are still elusive (Figure 7A). Importantly, trastuzumab has minor or no effects on the formation of heterodimers between ErbB2 and ligand-occupied ErbB partners. This process is mediated by subdomain II of ErbB2, the dimerization arm of the receptor.⁴ In addition, according to recent data, trastuzumab does not downregulate ErbB2 from the cell surface, at least in tumor cells.⁸ This information is crucial to understand the cardiac actions of trastuzumab. Indeed, in cardiomyocytes, ErbB signaling occurs at low levels of ErbB2 and is exclusively ligand-triggered (see

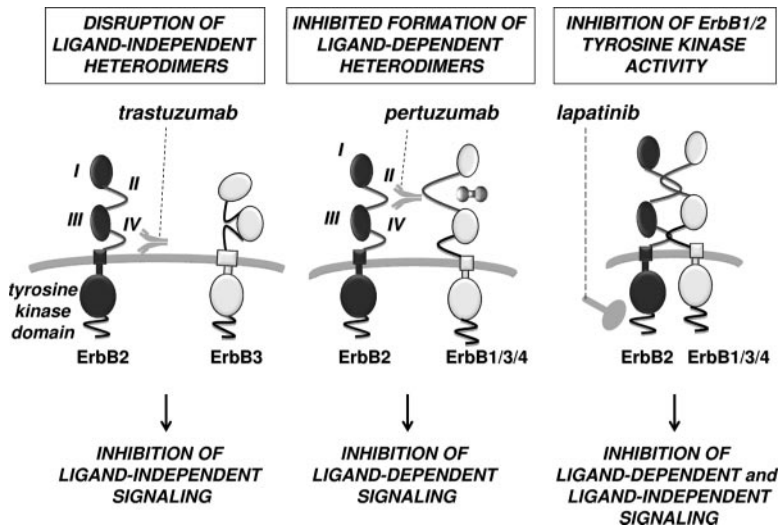


Figure 7. Mode of action of current ErbB2 inhibitors. Trastuzumab, pertuzumab, and lapatinib have a different mode of action on ErbB signaling. Trastuzumab (left) is a humanized monoclonal antibody to subdomain IV of ErbB2. This leads to disruption of ErbB2-ErbB3 complexes, formed when ErbB2 is overexpressed, and in absence of ligand-binding to ErbB3. The mechanisms of this disruption are still unclear. Disruption of these complexes inhibits PI3K signaling and Akt activation and explains the antiproliferative effects of trastuzumab in ErbB2-amplified tumor cells. Effects of trastuzumab on ligand-induced dimerization of ErbB receptors seem minor or absent. Pertuzumab (middle) is a humanized monoclonal antibody to subdomain II, the dimerization arm of ErbB2. Pertuzumab leads to inhibition of ligand-induced ErbB2 signaling, not of ligand-independent ErbB2 signaling. Lapatinib (right) is a small molecule tyrosine kinase inhibitor of ErbB1 and ErbB2. Lapatinib blocks tyrosine kinase activity, independently of whether this activity has been triggered by a ligand or not.

above). One may wonder, therefore, whether trastuzumab *per se* affects (ligand-induced) cardiac ErbB signaling in cardiomyocytes. To the best of our knowledge, there are no studies available that have directly looked into this question. Studies using other “trastuzumab-like anti-ErbB2 antibodies” (see below) should be interpreted cautiously because these antibodies may have a different mode of action than trastuzumab. Specific experiments with trastuzumab in cardiomyocytes are urgently awaited. Because of the humanized antigenic nature of trastuzumab these studies seem, however, only possible in human cardiomyocytes. These studies are a prerequisite to establish whether trastuzumab also fails to abrogate ligand-induced ErbB signaling in cardiomyocytes, similarly as it does in tumor cells. Trastuzumab may downregulate ErbB2 in cardiomyocytes, or could perhaps interfere with the transactivation of cardiac ErbB receptors by GPCRs. The latter would reflect an unanticipated “on-target” side effect of trastuzumab in the heart, and explain some of the paradoxes emerging in this field.

What can we learn from experiments in which trastuzumab-induced ventricular dysfunction has been directly studied or mimicked? There are only few cardiac biopsy studies of trastuzumab-induced cardiomyopathy in patients, and these have failed to provide crucial information.⁷¹ One study in mice⁷² has shown that trastuzumab induces cardiomyocyte apoptosis *in vitro*, and left ventricular dysfunction *in vivo*, which is a bit surprising given the humanized nature of trastuzumab. Unfortunately, this study did not assess whether trastuzumab-induced effects in the mouse heart were related to inhibition of ErbB signaling. A few other studies have looked at the effects of “trastuzumab-like anti-ErbB2 antibodies” on isolated cardiomyocytes. These studies have provided inconsistent results, varying from the sensitization to anthracycline/oxidative stress-induced apoptosis in presence of NRG-1,³¹ to the spontaneous induction of apoptotic cell death^{72–74} or the induction of myofilament structural damage without cell death.⁷⁵ Overall, it remains unclear to what extent the effects of these experimental antibodies specifically reflect trastuzumab-induced

ventricular dysfunction, because their mode of action is unknown or differs from that of trastuzumab.

What can we learn from the effects of other ErbB2 antagonists? ErbB2 antagonists can be divided in 2 classes, namely monoclonal antibodies such as trastuzumab and pertuzumab, and small molecule tyrosine kinase inhibitors such as lapatinib. Trastuzumab has been discussed in the previous paragraphs.

Lapatinib, a small molecule inhibitor directed against the phosphotyrosine kinase activity of ErbB1 and ErbB2, has been recently introduced as an alternative to, or as an add-on therapy with, trastuzumab.⁷⁶ In contrast to trastuzumab, lapatinib inhibits both constitutive and ligand-induced ErbB signaling (Figure 7C).^{77,78} We recently confirmed that lapatinib partly inhibits NRG-1-induced ErbB2 signaling in the ventricle. Based on this working mechanism, one may straight-forwardly anticipate that lapatinib affects ventricular function, as a direct result of its action on ErbB signaling in the heart (on-target toxicity). Current clinical trials, however, have reported that these side effects may be minor⁷⁹ (asymptomatic drops in left ventricular ejection fraction in 1.4% of patients, and symptomatic events in 0.2% of patients). These results may perhaps be explained by the “biased” design of these trials, including patients that have already supported trastuzumab therapy without cardiotoxic side effects, and by starting lapatinib treatment only late after anthracyclines.^{80,81} To what extent the reduced cardiotoxicity of lapatinib can also be explained by the fact lapatinib only partially blocks NRG-1/ErbB signaling (leaving the ErbB4-Akt axis unaffected) or by the activation of AMP-activated protein kinase, a key regulator in mitochondrial energy production pathways in human cardiac cells and cancer cells⁸² remains to be confirmed.

Second, pertuzumab, a humanized monoclonal antibody that binds to the dimerization domain of ErbB2 (subdomain II), is being tested in phase I and II trials for the treatment of ErbB2 amplified tumors. Different from the mode of action of trastuzumab, pertuzumab functions by blocking the association of ErbB2 with ligand-occupied ErbB members (Figure 7B).⁸³ From this working mechanism, a direct impact

on ligand-induced NRG-1/ErbB signaling in the ventricle is evident and on-target cardiac side effects expected. Consistently, ventricular dysfunction attributable to pertuzumab has been documented, although studies are small. In one phase II study of pertuzumab in prostate cancer, 27% of patients developed an asymptomatic decrease of left ventricular ejection fraction of at least 10 points.⁸⁴ In another small phase II trial that combined trastuzumab with pertuzumab, 6 of 11 patients experienced a significant reduction in left ventricular ejection fraction, of which one was severe and induced congestive heart failure symptoms.⁸⁵

In summary, given the cardioprotective effects of paracrine NRG-1/ErbB signaling in the heart, therapies that block ErbB signaling may be expected to induce ventricular dysfunction. These effects should be pronounced in conditions in which the injured or overloaded heart has progressed into a state of “ErbB signaling dependency.” Prior anthracycline-induced damage is clearly one of these conditions⁸⁶ but there may be other, and these should be better identified. Surprisingly, however, confirming the link between trastuzumab-induced cardiomyopathy and impaired paracrine NRG-1/ErbB signaling seems less evident than anticipated. Attempts to clarify this link should be pursued. Establishing the exact nature of trastuzumab-induced cardiomyopathy, especially in terms of “on-target” or “off-target” toxicity, is crucial for the design of future ErbB2 antagonists. The cardiotoxicity of other ErbB2 antagonists, like lapatinib and pertuzumab, whose mode of action is more directly linked to ligand-induced ErbB signaling, is only beginning to emerge. Overall, ErbB signaling in the heart is complex and delicate, and the precise mode of action of the anti-ErbB2 intervention will determine its impact on ventricular function. Hence, despite comparable efficacies on tumor cell Akt signaling and cancer growth, their impact on ligand-induced ErbB signaling in the heart may greatly vary. Hence, harmful effects of anti-ErbB2 therapeutics on ventricular function should not be universalized.

Concluding Remarks

From the point of view of a cardiologist, there are important lessons to be learned from the insights derived from the cardiac side effects of targeted anticancer therapy. The awareness that specific paracrine ligand-receptor interactions seem to be indispensable for normal cardiac performance and integrity contributes to the changing conceptual view on cardiac function. To end this review, we would like to incorporate this progress in a historical frame created in a recent article by Brutsaert.⁸⁷ As he explains, until the 19th century the ventricle was considered as part of a hydraulic input–output system with the ventricle itself as a black box. At the beginning of the 20th century, the ventricle was viewed as a hemodynamic pump, the function of which was described in pressure–volumes curves, with the cardiomyocyte as a black box. In the middle of the 20th century, the ventricle was viewed as a muscular pump, and its function became understood in principles of muscle physiology, both during contraction and relaxation. Ventricular noncardiomyocytes remained in the black box. Currently, one may view the heart as a pluricellular tissue pump in which crosstalk between different cell types and structural components (car-

diomyocytes, fibroblasts, endothelial cells, extracellular matrix, in situ stem cells) contribute to ventricular homeostasis. Interactions between ErbB receptors and their paracrine ligands are new players in this field. The vulnerability of the heart when this system is impaired is a new pathophysiological entity. Unraveling the physiological and molecular mechanisms that drive or disturb these intercellular networks contributes to a better comprehension of the cardiovascular system and its behavior in disease and will lead to new therapies.

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