Role of Neuregulin-1/ErbB Signaling in Cardiovascular Physiology and Disease

Implications for Therapy of Heart Failure

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Abstract—Since the discovery that neuregulin-1 (NRG-1)/ErbB signaling is indispensable in cardiac development, evidence has shown that this system also plays a crucial role in the adult heart. In patients, an inhibitory ErbB2 antibody, trastuzumab, used in the treatment of mammary carcinomas, increases the risk for the development of cardiotoxic cardiomyopathy. Postnatal disruption of NRG-1/ErbB signaling by gene targeting in mice leads to dilated cardiomyopathy. Initially, the search for the mechanisms behind these observations focused mainly on the effects of NRG-1 on cardiomyocyte growth and survival and revealed that NRG-1 has Akt-dependent antiapoptotic effects in cultured cardiomyocytes. In vivo studies, however, did not uniformly reinforce a role for apoptosis in the development of cardiomyopathy induced by impaired NRG-1/ErbB signaling. More recent studies have revealed that NRG-1 is involved in the regulation of cardiac sympathovagal balances by counterbalancing adrenergic stimulation of the adult myocardium and through an obligatory interaction with the muscarinic cholinergic system. NRG-1 is synthesized and released by the endocardial and cardiac microvascular endothelium, dynamically controlled by neurohormonal and biomechanical stimuli. The physiology of the cardiac NRG-1/ErbB system has implications for the treatment of both cancer and heart failure. Clinical studies in breast cancer with novel ErbB inhibitors are currently underway. Novel oncological indications for ErbB inhibition are emerging; cardiovascular side effects need to be carefully monitored. On the other hand, pharmacological activation of ErbB signaling is likely an unrecognized and beneficial effect of currently used drugs in heart failure and a promising therapeutic approach to prevent or reverse myocardial dysfunction. (***Circulation*. 2007;116:954-960.**)

Key Words: endothelium ■ receptors, ErbB-2 ■ heart failure ■ neuregulins

Neuregulin-1 (NRG-1) is a member of the epidermal growth factor (EGF) family known to activate proliferation, differentiation, and survival of many tissue types, including breast epithelial cells, glial cells, neurons, and myocytes.[1–4] Its biological effects are mediated by a set of tyrosine kinase receptors (ErbB2, ErbB3, and ErbB4) that dimerize on ligand binding, leading to phosphorylation and downstream signaling.[5] NRG-1 biology is complicated by the fact that multiple splice variants are produced from the NRG-1 gene (for review, see elsewhere[6]). These NRG-1 isoforms can be divided into 3 types. Type I and II NRGs contain an immunoglobulin-like domain and are single-pass transmembrane proteins. Type III NRGs, containing a cysteine-rich domain, are 2-pass transmembrane proteins. Proteolytic cleavage of type I and II NRGs by members of the a-disintegrin and metalloprotease (ADAM) family such as tumor necrosis factor-α converting enzyme (ADAM17) and meltrin-β (ADAM19)[7,8] results in the release of a bioactive fragment. Cleavage of type III isoforms generates a transmembrane N-terminal fragment (Figure 1).[9] A common motif to all NRG isoforms is the EGF-like receptor binding domain. Alternative splicing of this domain leads to α or β variants; the β isoform has been reported to be 10 to 100 times more active than the α variant.[6]

NRG-1/ErbB signaling is best known for its indispensable role during cardiac and neuronal development. It also has been implicated in the development of schizophrenia and several human cancers.[6,10] In fact, ErbB2, also known as HER-2 or c-neu, was initially discovered as an oncogene variant frequently overexpressed in many tumor types.[11] It was only by accident that it became evident that NRG-1 also is involved in heart failure, more specifically by the unforeseen "cardiotoxicity" of trastuzumab (Herceptin), an inhibitory antibody against ErbB2.[12,13] To date, multiple functions for NRG-1 in the developing and mature heart have been demonstrated[12–35] (Table 1). Originally, these functions reflected only the effects of NRG-1 on cell survival and growth in conditions of cell stress,[23–27] providing a possible explanation for the cardiotoxic effects of trastuzumab. Recently, more physiological functions of the NRG-1/ErbB system have been discovered, including the interaction with sympathovagal control systems of the heart.[28–30] This review

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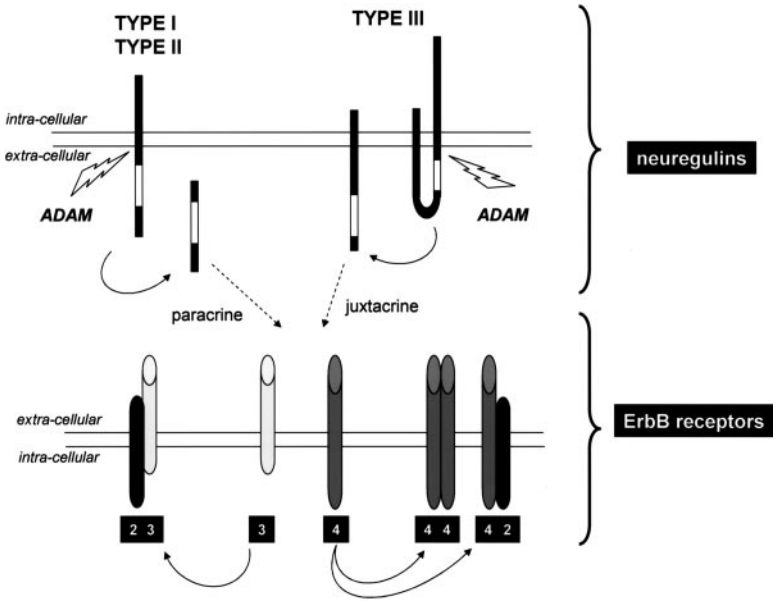

Figure 1. NRG-1/ErbB signaling. NRG-1 isoforms can be divided into 3 types. Type I and II NRGs are single-pass transmembrane proteins. Proteolytic cleavage by a member of the ADAM family results in the release of a bioactive fragment containing the EGF-like receptor binding domain. Type III NRGs have a cysteine-rich domain and are 2-pass transmembrane proteins. Cleavage of type III isoforms generates a transmembrane N-terminal fragment that includes the EGF-like receptor binding domain (symbolized as white fragments in the black NRG-1 bars). Therefore, type I and II isoforms are specialized for paracrine signaling, whereas type III NRGs serve as juxtacrine signals. Type I NRGs are the predominant, if not the only, type in the heart. NRG-1 can bind to 2 receptors: ErbB3 and ErbB4. Ligand binding induces formation of homodimers and heterodimers. ErbB3 homodimers are functionally defective because ErbB3 has impaired catalytic activity. ErbB2 cannot directly bind NRG-1, but it is the favored partner for heterodimerization. On dimerization, the intrinsic kinase domain is activated, resulting in phosphorylation of specific tyrosine residues within the cytoplasmic tail of the receptor and subsequent downstream signaling.

summarizes the most recent discoveries regarding NRG-1/ErbB signaling in cardiovascular physiology and disease and discusses implications for treatment of cancer and chronic heart failure.

Role of NRG-1 in the Fetal Heart

The first evidence for a function of the NRG-1/ErbB pathway in cardiac morphogenesis was revealed by studies of NRG-1–, ErbB2-, and ErbB4-null mice. NRG-1–null mice die midway through embryogenesis (10.5 days) as a result of the absence of normal trabeculation of the ventricles.[14] An NRG gene mutation that causes all transmembrane NRGs to have their tail truncated to a length of only 3 amino acids has the same cardiac phenotype as pan-NRG–null mice. This cytoplasmic tail-deleted mutant is resistant to proteolytic release of its extracellular domain, a process required for ErbB receptor activation. Thus, proteolytic processing of the membrane-bound NRG isoforms is critically controlled by their intracellular domain and is a crucial step in NRG-1/ErbB signaling.[15]

ErbB2- and ErbB4-null mice display a failure in ventricular trabeculation identical to that seen in NRG-1–null mice.[16,17] Similarities in the cardiac phenotype of these gene mutants suggest that ErbB2 and ErbB4 function as NRG-1 receptors in the fetal heart. Neither ErbB2 nor ErbB4 alone can compensate for the loss of the other receptor, suggesting that NRG-1 signaling in the heart requires ErbB2/ErbB4 heterodimers. In the fetal heart, NRG-1 is produced in the endocardial endothelium, and ErbB2 and ErbB4 are expressed on the nearby cardiomyocytes.[14,17,36] In contrast, the ErbB3 receptor is expressed in neither the endocardium nor the myocardium. It is detectable only in mesenchymal cells of the endocardial cushion, the structure that separates the embryonic atrium and ventricle. ErbB3-null mice exhibit cardiac cushion abnormalities, leading to reflux of blood through defective valves.[18,19] Interestingly, apart from its role in ventricular trabeculation and cardiac cushion formation, NRG-1 also converts embryonic cardiomyocytes into cells of the conduction system[20,21] and promotes differentiation and survival of cardiomyocytes derived from embryonic stem cells[22] (Table 1).

TABLE 1. Evidence for NRG-1/ErbB Functions in the Embryonic and Adult Heart

Function/Effect	Receptor	Reference
Embryonic		
Ventricular trabeculation	ErbB2/4	14–17
Valve formation	ErbB3	18, 19
Development of conduction system	NA	20, 21
Differentiation of cardiomyocytes	ErbB4	22
Adult (in vitro)		
Hypertrophy of cardiomyocytes	NA	23–25
Survival of cardiomyocytes	ErbB2/4	23–27
Modulation of myocardial contractility	NA	28
Modulation of muscarinic receptor signaling	NA	29, 30
Electrical/mechanical coupling of cardiomyocytes	NA	31
Adult (in vivo)		
Protection against toxic cardiomyopathy	ErbB2	12, 13
Protection against cardiomyopathy	ErbB2/4	32–35

NA indicates not assessed.

NRG-1 Promotes Survival and Growth of Cardiomyocytes In Vitro

In the adult heart, NRG-1 continues to be expressed in cardiac endothelial cells,[37] whereas ErbB2 and ErbB4, but not ErbB3, receptors are still expressed in cardiomyocytes.[23] More precisely, NRG-1 expression seems to be restricted to the endothelial cells near cardiomyocytes (in the endocardium and in the myocardial microvasculature) because it is absent in larger coronary arteries and veins and in aorta.[24] Various in vitro effects of recombinant NRG-1 on postnatal and adult

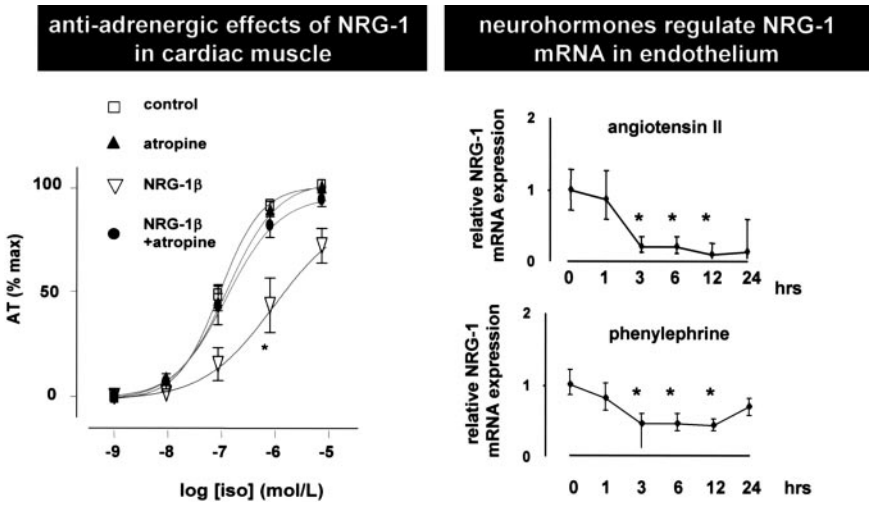

Figure 2. Relation between NRG-1 and the neurohormonal system. Left, NRG-1 influences β-adrenergic responsiveness of rabbit papillary muscles by shifting the dose-response curve to isoproterenol by 1 logarithmic unit to the right. This effect is completely inhibited by the muscarinic receptor blocker atropine. Peak twitch active tension (AT) is depicted as a function of isoproterenol (ISO) concentration in control conditions and after administration of atropine, NRG-1β, and NRG-1β plus atropine. *$P<0.05$ for EC50 in treatment vs control. Right, Neurohormonal stimuli influence NRG-1 expression in cultured cardiac endothelial cells. NRG-1 mRNA expression decreases in response to angiotensin II and phenylephrine (10 μmol/L). *$P<0.05$ vs control. Modified from Lemmens et al,[24] with permission of the publisher. Copyright © 2006, American Society for Biochemistry and Molecular Biology.

cardiomyocytes have been demonstrated, including the regulation of survival,[23,26] hypertrophy,[23,25] proliferation,[23,25] myofibrillar organization,[38] and cell-to-cell contact between cardiomyocytes.[31] In addition, we and others have shown that NRG-1 released from endothelial cells protects cultured cardiomyocytes from apoptotic cell death induced by oxidative stress and anthracyclines.[24,27] Although initially postulated to be mediated by ErbB4 signaling,[26,27] we recently showed that these cell-protective effects also are mediated by ErbB2.[24] These findings may have implications for the clinical use of inhibitory ErbB2 antibodies in patients. Although ErbB2 is not able to bind NRG-1 directly, its biological importance is explained by the fact that it is the preferred partner for ErbB3 and ErbB4 for heterodimerization and essential for ErbB signaling in the adult heart.[39]

Signaling pathways activated downstream of ErbB include ERK1/2 and PI3-kinase/Akt. The hypertrophic response to NRG-1 relies mainly on ERK 1/2 activation, whereas the antiapoptotic effects are more likely to be Akt dependent.[25,26] More recently, ErbB2-dependent activation of focal adhesion kinase has been associated with restoration of cell-to-cell contact between isolated myocytes, which suggests a possible role for NRG-1 in the maintenance and repair of electrical and mechanical coupling in cardiomyocytes.[31]

Interaction Between NRG-1/ErbB Signaling and the Neurohormonal System

Apart from playing a protective role in myocardial tissue integrity, the cardiac NRG-1/ErbB system interacts, according to recent evidence, with cardiovascular neurohormonal autoregulatory systems. Most important, as shown in Figure 2, NRG-1 diminishes the inotropic myocardial response to adrenergic stimulation by shifting the dose-response curve of isolated cardiac muscles to isoproterenol almost by 1 logarithmic unit to the right,[28] thereby mimicking the antiadrenergic effects of muscarinic cholinergic receptor signaling. Interestingly, antiadrenergic NRG-1/ErbB signaling and antiadrenergic muscarinic signaling rely on mutual cooperation. Indeed, antiadrenergic NRG-1/ErbB signaling disappears when muscarinic cholinergic receptor is blocked (Figure 2, left),[28] and antiadrenergic muscarinic cholinergic signaling is diminished in the absence of NRG-1.[29]

Within this cooperation between the ErbB and muscarinic receptor signaling, adaptive regulation of NRG-1 synthesis and release from cardiac endothelial cells seem to be important. Indeed, synthesis and release of this factor are controlled, at least in part, by the activity of the adrenergic and renin-angiotensin systems. This is illustrated in Figure 2 (right), which shows how angiotensin II and phenylephrine directly downregulate NRG-1 expression in cultured endocardial and cardiac microvascular endothelial cells,[24] the main source of NRG-1 in the heart.

A possible new role of NRG-1/ErbB signaling in cardiovascular homeostasis, as suggested from these observations, is summarized in Figure 3. From our in vitro findings, we speculate that, through its cooperation with the cholinergic system for antiadrenergic effects, NRG-1 can decrease cardiac output and hence blood pressure. By sensing levels of circulating angiotensin II and epinephrine in the blood, released in conditions of low arterial blood pressure, the cardiac endothelium adapts NRG-1 synthesis and hence fine-tunes this antiadrenergic effect according to peripheral needs. This interesting new conjecture needs to be further validated in vivo. For example, it would be interesting to see whether sympathetic tone is increased in patients treated with trastuzumab and in NRG-1/ErbB–deficient mice.

The molecular mechanisms underlying the cooperation between the NRG-1/ErbB system and the cholinergic system are still under investigation. We have recently reported that the antiadrenergic effect of NRG-1 is mediated by nitric oxide (NO) synthesized by endothelial NO synthase (eNOS) in cardiomyocytes.[28] This mechanism is consistent with the described effects of NO on myocardial β-adrenergic signaling in cardiomyocytes with genetically deleted or overexpressed eNOS.[40,41] Interestingly, acetylcholine also relies on postsynaptic activation of eNOS in cardiomyocytes for attenuation of β-adrenergic myocardial stimulation.[42,43] Thus, both NRG-1 and the parasympathetic system need eNOS to exert antiadrenergic effects, providing a molecular link between the 2 pathways. To what extent activation of eNOS explains the

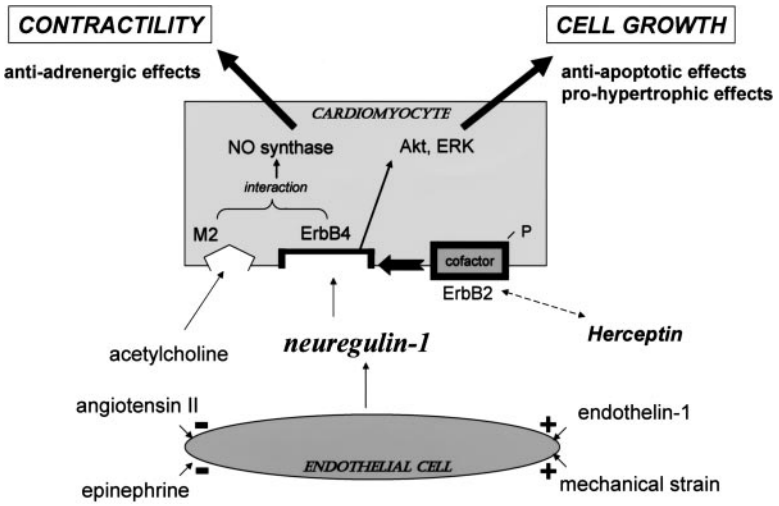

Figure 3. Source and actions of NRG-1 in the heart. NRG-1 is synthesized in endothelial cells near cardiomyocytes (in the endocardium and myocardial microvasculature). Endothelial synthesis is suppressed by the blood pressure–enhancing hormones angiotensin II and epinephrine and stimulated by endothelin-1 and mechanical strain. On release, NRG-1 binds to ErbB4 on cardiomyocytes that, after homodimerization with ErbB4 or heterodimerization with phosphorylated ErbB2, leads to activation of Akt and ERK, with subsequent growth-promoting and cell-protective actions. In addition, cooperative activities with the muscarinic receptor (M2) and the activation of cardiomyocyte NO synthase affect myocardial contractility and adrenergic responsiveness.

cooperation between ErbB and muscarinic signaling is, however, still unclear.

NRG-1/ErbB Signaling During the Progression of Chronic Heart Failure

Together with the cardiotoxic effects of trastuzumab in patients, the premature development of dilated cardiomyopathy on pressure overload in NRG-1/ErbB–deficient mice[32–35] (Table 2) raises the hypothesis that NRG-1 plays a prominent role in the pathogenesis of chronic heart failure (CHF). Animal models have demonstrated important changes within the cardiac NRG-1/ErbB system during the progression of CHF.[24,44,45] Interestingly, when these changes are depicted on a time axis, NRG-1/ErbB expression first rises in the early stages of the disease and declines only in the later stages when pump failure occurs (Figure 4). The initial robust increase in NRG-1 mRNA in the left ventricle occurs during development of concentric left ventricular hypertrophy and is most likely the result of mechanical wall strain. The subsequent decline in NRG-1 expression coincides with the development of eccentric ventricular hypertrophy and pump failure and is accompanied by a downregulation in the mRNA levels of ErbB2 and ErbB4. The mechanisms of NRG-1 mRNA downregulation during pump failure are perhaps related to the increased levels of angiotensin II and epinephrine, both of which reduce NRG-1 mRNA synthesis in cardiac endothelium.[24] To what extent these changes in NRG-1 and ErbB mRNA expression ultimately lead to alterations in cardiac NRG-1/ErbB signaling is currently under investigation. Activation of NRG/ErbB signaling in the myocardium at the early stages of CHF would be adaptive in terms of myocardial tissue integrity and growth and as a counterbalance to exaggerated adrenergic activation. Inactivation of NRG-1/ErbB signaling at later stages may also be adaptive, at least in terms of hemodynamic conditions of the peripheral circulation, in that it should increase adrenergic stimulation of the failing ventricular pump. The concomitant loss of NRG-1–

TABLE 2. Adult Mouse Models With Deficient NRG-1/ErbB Signaling

Genotype and Cardiac Phenotype	Reference
ErbB2 CKO	
Dilated cardiomyopathy	32, 33
Increased mortality on pressure overload	32, 33
Increased sensitivity to anthracyclines of cardiomyocytes in vitro	32
ErbB4 CKO	
Dilated cardiomyopathy	34
NRG-$1^{+/-}$	
Exacerbation of doxorubicin-induced heart failure	35

CKO indicates conditional knockout, postnatal mutation in ventricular cardiomyocytes; NRG-$1^{+/-}$, heterozygous knockout of NRG-1.

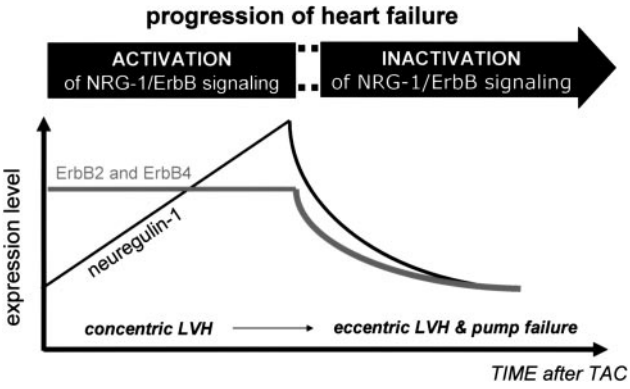

Figure 4. Role of NRG-1 in the pathogenesis of heart failure. Transaortic constriction (TAC) in rat slowly increases the levels of NRG-1 mRNA in the myocardium, followed by normalization to initial levels on transition to eccentric hypertrophy and pump failure. The initial increase in left ventricular NRG-1 expression is most likely the result of mechanical wall strain. The subsequent downregulation of NRG-1 expression during left ventricular dilation and pump failure is presumably mediated by enhanced circulating levels of angiotensin II and epinephrine. At the same time, myocardial ErbB2 and ErbB4 receptors also are severely downregulated.[44] Activation of NRG/ErbB signaling in the myocardium at the early stages of the disease would be adaptive in terms of myocardial tissue integrity and growth and as a counterbalance to exaggerated adrenergic activation. Inhibition of NRG-1/ErbB signaling at later stages most likely is an attempt to increase the compensatory action of the adrenergic system during pump failure. LVH indicates left ventricular hypertrophy.

mediated tissue protection, however, may be detrimental and a crucial step in the further remodeling process of the ventricle.

Given these time-dependent changes in NRG-1/ErbB signaling in the progression of CHF and the downregulating activities of angiotensin on NRG-1 mRNA synthesis, it is tempting to speculate that the beneficial actions of angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists in CHF may, to some extent, be explained by restoring NRG-1 synthesis in the failing heart. Vice versa, given the robust inducing effect of endothelin-1 on NRG-1 expression and release in the cardiac endothelium,[24] it is possible that the disappointing results of endothelin receptor antagonists in the treatment of CHF are related to an unforeseen and detrimental downregulation of cardiac endothelial NRG-1 activity.[24]

Stimulation and Inhibition of NRG-1/ErbB Signaling: Pharmacological Effects on the Heart In Vivo

Inhibition of cardiac ErbB2 signaling in vivo leads to cardiomyopathy in the presence of anthracyclines or pressure overload but also in the apparent absence of any stress factor on the heart. Trastuzumab initially caused cardiac dysfunction in up to 7% of patients when used as a single agent and in up to 27% when combined with anthracyclines. Severe CHF, New York Heart Association class III and IV, occurred in 16% of patients treated with a combination of trastuzumab and anthracyclines.[46] More recent studies incorporating well-designed prospective cardiac monitoring suggest a lower incidence of symptomatic CHF (up to 4% for the trastuzumab-anthracycline combination). Nevertheless, asymptomatic cardiac dysfunction still occurs in >14% of patients receiving trastuzumab with anthracyclines and in 7% receiving trastuzumab alone.[47]

Despite extensive research, cardiotoxic effects of trastuzumab, and ErbB2 inhibition in general, have remained difficult to explain. Trastuzumab cardiotoxicity appears to be dose independent and largely reversible, suggesting a different mechanism from that of anthracyclines.[48] On the basis of the observation that anthracyclines increase the cardiotoxic effects of trastuzumab and promote the onset of left ventricular dysfunction in NRG-1 or ErbB gene deletion in mice, a 2-hit model for trastuzumab cardiotoxicity has been proposed in which an initial loss of ErbB2-dependent survival pathways in cardiomyocytes promotes subsequent cardiotoxic effects of anthracyclines. Multiple in vitro studies support this reasoning by showing that interference with ErbB2 signaling promotes a proapoptotic cascade in cardiomyocytes and inhibits prosurvival pathways.[49–51] However, in vivo studies have failed to uniformly reinforce a role for apoptosis in the development of cardiomyopathy in NRG-1/ErbB-deficient mice. Indeed, whereas Crone et al[32] detected apoptotic cell death in cardiac-specific ErbB2 knockouts, other groups did not observe myocardial apoptosis in NRG-1/ErbB–deficient mice.[33–35] Therefore, other aspects of NRG-1 signaling, perhaps related to its interaction with the neurohormonal system, are likely involved.

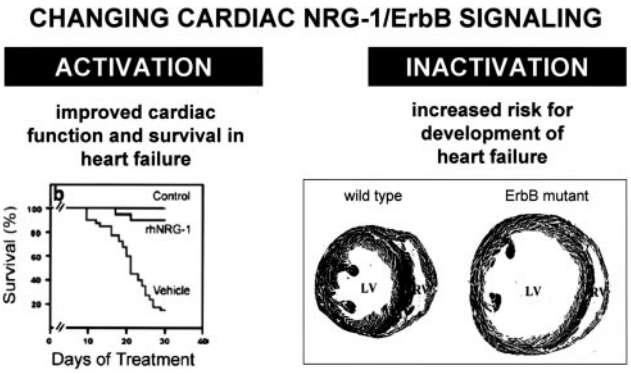

Figure 5. Effects of activation and inactivation of NRG-1/ErbB signaling on cardiac function. Inactivation of NRG-1/ErbB signaling by gene targeting in mice can lead to heart failure and increased susceptibility to anthracycline cardiotoxicity (right; modified from Crone et al,[32] by permission from Macmillan Publishers Ltd, copyright © 2002). Activation of ErbB signaling by recombinant NRG-1 improves survival of chronic heart failure in rats (eg, after myocardial infarction) (left; modified from Liu et al,[52] with permission of the publisher. Copyright © 2006, American College of Cardiology Foundation). Recombinant human NRG-1 (rhNRG-1) was administered intravenously starting 1 week after the infarct and was given once daily for 5 days.

Following the notion that NRG-1/ErbB signaling has protective effects on the myocardium and is inhibited on transition to pump failure, the question arises whether pharmacological ErbB activation has any preventive or curative potential in heart failure. In a very elegant study, Liu and colleagues[52] showed that short-term intravenous administration of recombinant NRG-1 improves cardiac function and survival in different models of cardiomyopathy, including toxic, ischemic, dilated, and viral cardiomyopathy (Figure 5). Interestingly, the benefits of NRG-1 treatment appeared to be additive to those of angiotensin-converting enzyme inhibitors and remained present when treatment was started after the onset of cardiomyopathy. This study suggests that NRG-1/ErbB activation may be a novel and powerful therapeutic approach for heart failure treatment. However, possible side effects that might be feared when a growth factor is used systemically on a long-term basis should be investigated carefully.

Conclusions

In addition to its crucial role during cardiac development, the NRG-1/ErbB system continues to play an important role in adult cardiac physiology. Initially, NRG-1 has been viewed only as a promoter of myocardial growth and survival. New experimental evidence now suggests that NRG-1 regulates myocardial performance and sympathovagal balance and that it dynamically participates in the hemodynamic homeostasis of the cardiovascular system. These multiple aspects of NRG-1 signaling should help us to understand the role of NRG-1 in cardiovascular physiology and to predict cardiac consequences of NRG-1 targeting. Whereas inhibition of ErbB signaling is a powerful treatment for mammary and perhaps other carcinomas, activation of ErbB signaling is emerging as a novel pharmacological approach to treat CHF.

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Disclosures

None.

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