Neuregulin in Cardiovascular Development and Disease

Oghenerukevwe Odiete, Michael F. Hill, Douglas B. Sawyer

Abstract: Studies in genetically modified mice have demonstrated that neuregulin-1 (NRG-1), along with the erythroblastic leukemia viral oncogene homolog (ErbB) 2, 3, and 4 receptor tyrosine kinases, is necessary for multiple aspects of cardiovascular development. These observations stimulated in vitro and in vivo animal studies, implicating NRG-1/ErbB signaling in the regulation of cardiac cell biology throughout life. Cardiovascular effects of ErbB2-targeted cancer therapies provide evidence in humans that ErbB signaling plays a role in the maintenance of cardiac function. These and other studies suggest a conceptual model in which a key function of NRG-1/ErbB signaling is to mediate adaptations of the heart to physiological and pathological stimuli through activation of intracellular kinase cascades that regulate tissue plasticity. Recent work implicates NRG-1/ErbB signaling in the regulation of multiple aspects of cardiovascular biology, including angiogenesis, blood pressure, and skeletal muscle responses to exercise. The therapeutic potential of recombinant NRG-1 as a potential treatment for heart failure has been demonstrated in animal models and is now being explored in clinical studies. NRG-1 is found in human serum and plasma, and it correlates with some clinical parameters, suggesting that it may have value as an indicator of prognosis. In this review, we bring together this growing literature on NRG-1 and its significance in cardiovascular development and disease. (Circ Res. 2012;111:1376-1385.)

Key Words: cardiac development ■ ErbB2 ■ ErbB3 ■ ErbB4 ■ heart failure ■ heregulin ■ heart failure therapy

In 2012, there are 2 forms of recombinant neuregulin (NRG-1β) being examined as potential therapies for heart failure. It is unlikely that the cardiovascular effects of NRG-1 were anticipated by the early investigators who cloned gene products of the NRG-1 gene and later inactivated NRG-1 in mice. Neuroscientists were exploring the developmental requirement of NRG-1 and erythroblastic leukemia viral oncogene homolog (ErbB) receptors in the central nervous system when they discovered that not only NRG-1 but also ErbB2 and ErbB4 receptors were critical to normal cardiac development. During the past 17 years since those publications, interest in the role of NRG-1/ErbB signaling in the heart has grown steadily fueled in part by the observations of oncologists developing ErbB2-targeted therapies for cancer. A growing body of literature suggests diverse functions for NRG-1/ErbB signaling in the adult cardiovascular system. In this review, we bring together this growing literature and identify important gaps that require further investigation.

Overview of NRG and ErbB Receptor Biology

The NRG-1 gene is a member of the epidermal growth factor (EGF) gene family. There are 4 structurally related genes encoding NRGs (NRG-1, NRG-2, NRG-3, and NRG-4), with NRG-1 being the most abundant in the cardiovascular system. Alternative splicing results in multiple isoforms of NRG-1, all of which share the common EGF domain that is essential for NRG-1 ligand function. Alternative splicing at the C-terminal of the EGF domain of NRG-1 leads to α- and β-variants that determine receptor affinity. NRG-1 gene products can be further subdivided into 3 types (types I, II, and III). Type I NRG-1 proteins are type I transmembrane proteins with a C-2 immunoglobulin-like (Ig) domain. Type II NRG-1 proteins express an N-terminal secretory signal peptide, a matrix-interacting kringle, and immunoglobulin domains, and are truncated after the β-domain, resulting in an active ligand on secretion. Type III NRG-1 proteins are almost exclusively expressed in neuronal cells and have a cysteine-rich domain that binds membranes and creates a tethered ligand. In type I NRG-1 proteins, the active form of NRG-1 is released after proteolytic cleavage at the juxtamembrane region lying on the carboxyl-terminal side of the EGF-like domain. The cleavage of type I NRG-1 is essential for its function and it is catalyzed by 3 type I transmembrane proteases: tumor necrosis factor-α-converting enzyme (also known as a disintegrin and metalloprotease 17); β-site of amyloid precursor protein–cleaving enzyme (also known as memapsin 2); and meltrin β (also known as a disintegrin and metalloprotease 19). NRG-1 exerts its effect in a paracrine manner via the ErbB family of tyrosine kinase receptors (ErbB2, ErbB3, and ErbB4). ErbB receptors consist of an extracellular ligand-binding domain, a transmembrane domain, and a cytoplasmic...
tyrosine kinase domain. NRG-1 binds to ErbB3 and ErbB4 (but not ErbB2) and induces a conformational change in ErbB3 or ErbB4 that allows dimerization with ErbB2 or ligand-activated ErbB3 or ErbB4 receptors. Intracellular kinase domains subsequently phosphorylate the intracellular C-terminal domain of the dimerization partner, providing docking sites for various adaptor proteins, such as SHC-adaptor protein, growth factor receptor-bound protein 2, and the regulatory subunit of phosphatidylinositol-3-kinase (PI3K), and downstream signaling that mediates several processes in many tissues that are time- and context-dependent.

NRG/ErbB Signaling Regulates Multiple Aspects of Cardiac Development

Specific disruption of NRG-1 or the ErbB2, ErbB3, or ErbB4 receptors in genetically altered mice leads to failure of cardiac development (Figure 1). Mice with disrupted NRG-1, ErbB2, or ErbB4 expression die at days 9 to 10 in utero with many defects, including lack of ventricular trabeculation, a process necessary for development of a full-thickness ventricular wall.2,9,10 Mice expressing a kinase-dead ErbB2 showed similar embryonic defects.11 At this stage of development, NRG-1 acts on myocyte signaling that mediates several processes in many tissues that are time- and context-dependent.

NRG-1/ErbB signaling is necessary for multiple aspects of cardiac development (Figure 2). The first evidence for this was found in humans during the development of ErbB2-targeted (also known as human epidermal growth factor receptor-2/neu) therapies for cancer. Patients with metastatic breast cancer receiving the humanized monoclonal anti-ErbB2 antibody trastuzumab alone or in combination with anthracyclines were found to have a high incidence of heart failure and left ventricular systolic dysfunction.21 In vitro and in vivo work has since developed several possible mechanisms for this observation.

Conditional cardiac myocyte–specific disruption of ErbB222,23 and ErbB424 has been examined in mice expressing myosin heavy chain promoter–regulated Cre-recombinase and floxed-ErbB receptors. These mice survive to birth but have development of spontaneous dilated cardiomyopathy, with increased susceptibility to stresses such as pressure overload and cardiotoxic anthracycline antibiotics. Ultrastructure analysis shows excess mitochondria and thin myofilaments.23 ErbB2 conditional mutant mice displayed a lengthened ventricular repolarization time, suggesting some impairment in ErbB2/ErbB4 heterodimers to mediate ventricular myocyte proliferation as well as differentiation, regulating the progression from a single-cell layer of ventricular myocardium to the formation of trabeculae. Interestingly, NRG-1/ErbB signaling also is critical for the development of the cardiac conduction system. NRG-1/β stimulates differentiation of embryonic cardiomyocytes into cells of the cardiac conduction system, with an increase in pacemaker current density, and transforms fetal cardiomyocytes into cardiac pacemaker-like cells.20 The concurrent events that determine whether NRG-1 stimulation drives a cell toward contractile vs conduction phenotype are yet unknown.

NRG-1/ErbB Signaling in Adult Heart Structure and Function

NRG-1/ErbB signaling has a role in the postnatal/adult heart that is distinct from the prenatal/embryogenesis stages of cardiac development (Figure 2). The first evidence for this was found in humans during the development of ErbB2-targeted (also known as human epidermal growth factor receptor-2/neu) therapies for cancer. Patients with metastatic breast cancer receiving the humanized monoclonal anti-ErbB2 antibody trastuzumab alone or in combination with anthracyclines were found to have a high incidence of heart failure and left ventricular systolic dysfunction.21 In vitro and in vivo work has since developed several possible mechanisms for this observation.

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the conduction system as well. Cardiac myocytes from conditional ErbB4 deletion mice exhibit both delayed conduction and impaired contractility.

One interpretation of these findings is that the NRG-1/ErbB system is required for physiological adaptation of the heart to changes in cardiac demand. This concept is supported by studies examining NRG-1/ErbB signaling during cardiac adaptation to pregnancy. During pregnancy in mice, left ventricular NRG-1/ErbB signaling is activated. Suppressing ErbB signaling with an ErbB1/2 tyrosine kinase inhibitor during pregnancy was associated with increased ventricular dilation and decreased systolic function, with premature death of pregnant mice. These studies collectively demonstrate the importance of an intact NRG-1β/ErbB signaling axis for adaptation of cardiac structure and function.

In vitro studies have demonstrated likely cellular mechanisms of how NRG/ErbB signaling may regulate cardiac adaptation to stress. This work collectively supports the concept that this is a paracrine and juxtacrine system that regulates the interaction between microvascular endothelial cells and cardiac myocytes (Figure 3). Multiple isoforms of NRG-1 are expressed in microvascular endothelial cells. Because these are almost entirely type I pre-NRGs, proteolytic activation is required for NRG-1 release from endothelial cells as an active ligand. Mild oxidative stress activates NRG-1 release and activity in vitro via a member of the matrix metalloproteinase family, as predicted by work in neuronal cell systems. Other physiological stimuli implicated in the regulation of NRG-1 expression and activity include angiotensin and phenylephrine, as well as endothelin. Although multiple NRG-1 isoforms are expressed in cardiac microvascular endothelial cells, it is yet unclear what role these play in the heart.

ErbB2 and ErbB4 are expressed in cardiac myocytes of the adult heart. ErbB3 expression also has been demonstrated in the adult myocardium, although its function has not been fully examined. Recombinant NRG-1β activates ErbB2 and ErbB4 receptor phosphorylation in isolated cardiac myocytes, with activation of protein kinase B (Akt)/PI3K, MEK/extracellular signal-regulated kinase, Src/focal adhesion kinase, and NO synthase, all of which have been linked to cellular responses, including cell survival, mitochondrial function, proliferation, growth, glucose uptake, sarcoplasmic reticulum calcium uptake, and focal adhesion formation. NRG-1β also regulates adrenergic responses in cardiac myocytes, having a negative inotropic effect in isolated rabbit papillary muscles, and protects myocytes from β1-adrenergic receptor–induced cell death. NRG-1β also regulates muscarinic cholinergic receptor expression and activity. Whether these antiadrenergic effects account for the deleterious effects of trastuzumab on cardiac function are yet to be fully examined.

Examination of what happens to cardiac myocytes when ErbB receptor expression and activity are disrupted in cell culture suggests other potential mechanisms for trastuzumab–associated cardiac dysfunction. Treatment of adult myocytes with an antibody to extracellular domain of ErbB2 causes early activation of extracellular signal-regulated kinase 1/2 that is likely caused by activation of ErbB2-ErbB2 dimers and downstream ErbB2–specific signaling while increasing myocyte sensitivity to anthra cyclines, leading to myofilament disorganization in adult myocytes.

NRG-1/ErbB activity interacts with myocardial metabolism, at least in vitro. NRG-1β induces cardiac myocyte glucose uptake in a PI3K–dependent manner to a similar magnitude as insulin. Interestingly, excess saturated fatty acid exposure, at least in vitro, induces a form of NRG resistance similar to the well-established and clinically important insulin resistance of type II diabetes mellitus. Treatment of cardiac myocytes with the saturated fatty acid palmitate seems to prevent ErbB coupling to the antiapoptotic PI3K/Akt pathway. These in vitro effects are suppressed by small amounts of the

Figure 2. Biological and physiological role of neuregulin-1 signaling in adult heart. Tissue activities known to be regulated by NRG-1/ErbB signaling in the adult cardiovascular system are shown (see text for details). EPC indicates endothelial progenitor cell.
monounsaturated fatty acid oleate. It is interesting to consider whether disruption of NRG-1/ErbB signaling, like insulin signaling, is a general phenomenon that contributes to end-organ damage in the setting of type II diabetes mellitus.

Metabolic stress, as seen in ischemic heart disease and chronic heart failure, also disrupts NRG-1/ErbB signaling via effects on heat shock protein 90 chaperone activity. Heat shock protein 90 requires ATP for functional protection of client proteins, including ErbB2.40 Inhibition of glycolysis or mitochondrial respiration in isolated myocytes causes heat shock protein 90 to rapidly dissociate from ErbB2.41 This, in turn, leads to degradation of ErbB2 and reduced NRG activation of extracellular signal-regulated kinase and Akt signaling. This phenomenon may account for reduced sensitivity of cardiac myocytes to NRG-1β under conditions of combined treatment with β-adrenergic receptor activation and electric pacing36 or of the intact heart late after myocardial infarction.42 Myocardial responsiveness to endogenous or exogenous NRG-1β is likely to be a function of these and other effects on receptor expression and stability.

**NRG Regulation of Vascular Biology and Angiogenesis**

The developmental role of NRG-1/ErbB signaling described has focused a lot of attention on its role in cardiac myocytes. In addition, NRG-1 seems to play an important role in regulating the structure and function of the vasculature in many organs. Both NRG-1 and ErbB receptors are expressed in vascular endothelial cells, and stimulation of endothelial cells in vitro or in vivo induces an angiogenic response similar to and independent of vascular endothelial growth factor.43 NRG-1 also can induce angiogenesis via enhanced expression of vascular endothelial growth factor.44 The ErbB3/4 ligands β-cellulin and amphiregulin have been implicated in the recruitment of vascular smooth muscle cells necessary for arteriogenesis.45,46 These findings suggest that one mechanism for the antitumor effect of ErbB2-targeted cancer therapies may be inhibition of angiogenesis.47 The concept that NRG-1 regulates angiogenesis in the setting of tissue ischemia recently has been demonstrated in a model of hindlimb ischemia.48

Endothelial progenitor cells (EPCs) originating in the bone marrow play a role in tissue repair after ischemic injury.49 Stimuli such as ischemic injury and exercise, which have been shown to activate tissue NRG-1,50 are also potent inducers of EPC mobilization and recruitment to the heart and skeletal muscle.50,51 It is interesting that EPCs express ErbB2 and ErbB3, and NRG-1 regulates EPC biology, with effects on survival as well as induction of differentiation signaling.52 The extent to which NRG-1 modulation of EPC function is involved in myocardial recovery after ischemic injury,

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**Figure 3. Neuregulin-1 signaling in cardiac myocytes.** NRG-1 is expressed in the microvascular endothelium, whereas ErbB2 and ErbB4 are expressed in ventricular cardiac myocytes. NRG-1 activates several distinct and interacting signaling cascades, including extracellular signal-regulated kinase (Erk)1/2, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt), and Src/focal adhesion kinase (FAK), leading to downstream changes in myocyte function (see text for details). EPC indicates endothelial progenitor cell; ROS, reactive oxygen species; eNOS, endothelial NO synthase; PKG, protein kinase G; PLN, phospholamban; SERCA, sarcoplasmic reticulum calcium ATPase.
ischemia-induced angiogenesis, and the beneficial effects of exercise warrant further investigation.

**NRG in the Central and Peripheral Nervous System: Implications for Cardiovascular Function**

NRG-1 and ErbB receptors are distributed extensively in the central and peripheral nervous systems.53,54 The biological function of NRG-1/ErbB signaling in the nervous system is similar to that seen in the cardiovascular system: it is required for proper tissue organization and maturation in development, is activated in response to injury, and is a mediator for repair/recovery (Table 1). NRG-1 and its ErbB receptors are upregulated in the central nervous system after ischemia/injury, and administration of exogenous NRG-1β exerts a neuroprotective effect.55 Pretreatment with exogenous NRG-1β before cerebral ischemia and reperfusion resulted in significant improvement in the recovery of neurological function in rats, as well as infarct volume reduction.56,57 NRG-1 and its receptors are upregulated after traumatic brain injury in rat models.58,59 NRG-1β exerts a cytoprotective effect against oxidant injury in multiple cell types in the brain, including the microvascular endothelial cell.60

Some recent findings suggest that central nervous system NRG-1/ErbB signaling plays an important role in cardiovascular homeostasis. The rostral ventrolateral medulla of the brainstem is 1 of the most important regions involved in the central regulation of vasomotor tone. Injection of recombinant NRG-1β into the rostral ventrolateral medulla in rats lowered blood pressure, heart rate, and renal sympathetic nerve activity.61 Suppressing ErbB expression with small interfering RNA had the opposite effect, suggesting that baseline NRG-1/ErbB signaling modulates rostral ventrolateral medulla sympathetic tone. Whether these intriguing findings translate to new mechanisms for blood pressure control and hypertension warrants further investigation.

**NRG in Skeletal Muscle: Mediator of Cardiovascular Benefits of Exercise?**

NRG-1 and the ErbB receptors are important determinants of skeletal muscle biology, and this may have implications for cardiovascular function. NRG-1β induces myogenesis62 and myoblast differentiation.63 NRG-1 and ErbB receptors localize to the neuromuscular junction in skeletal muscle, where activation induces expression of postsynaptic acetylcholine receptor expression.64-66 Conditional knockout of ErbB2 in skeletal muscle leads to multiple defects in muscle, including decreased numbers of muscle spindles, impaired regeneration after injury, and defects in proprioception.67 Skeletal muscle NRG-1/ErbB signaling is activated in animals acutely by exercise,68 leading to the hypothesis that NRG-1β may mediate many of the effects of exercise (growth, glucose homeostasis) on skeletal muscle. In this context, it is interesting that NRG-1β stimulates skeletal muscle glucose uptake at comparable levels and is additive to the effects of insulin.69 This effect is mediated via PI3K/Akt/Akt-dependent translocation of glucose transporters (GLUT) to muscle cell membrane. Exercise training is known to improve glucose homeostasis, with clear benefits to cardiovascular function. Skeletal muscle ErbB3 expression was significantly increased in skeletal muscle in response to a progressive resistance training intervention in healthy adults.70 Fitness in healthy humans is associated with higher levels of circulating NRG-1β.70 Collectively, these findings lead us to speculate that skeletal muscle NRG-1β is an exercise hormone with actions at a distance, including the heart.

**Alteration of ErbB Receptor Expression in Cardiac Pathology**

NRG-1/ErbB signaling and expression have been studied in several cardiovascular disease states with interesting pathophysiological implications. In the setting of pressure overload induced by aortic banding in mice, expression of ErbB2 and ErbB4 mRNA levels in the left ventricle increase early during compensatory cardiac hypertrophy.71 After the transition to heart failure, however, myocardial ErbB2/ErbB4 mRNA and protein levels are decreased compared with control hearts. In diabetic animals, myocardial NRG-1 protein expression was significantly increased in skeletal muscle in response to the hypoxia-inducible factor-1α (HIF-1α) upregulation of ErbB2.72 In ventricular tissue from patients with advanced heart failure, increased NRG-1 and decreased ErbB2/ErbB4 mRNA expression have been observed.73 Based on the deleterious effects of conditional deletion of ErbB2

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**Table 1. Therapeutic Application and Effect of Recombinant NRG-1 in Noncardiac Adult Tissue**

<table>
<thead>
<tr>
<th>Author</th>
<th>Formulation of NRG-1</th>
<th>Tissue</th>
<th>Pathology</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marchionni et al</td>
<td>rhGGF2</td>
<td>CNS</td>
<td>Encephalomyelitis (mouse model of multiple sclerosis)</td>
<td>Delayed signs, decreased severity, reduction in relapse rate, and increased remyelination</td>
</tr>
<tr>
<td>Xu et al</td>
<td>rhNRG-1</td>
<td>CNS</td>
<td>Acute ischemic stroke (transient occlusion)</td>
<td>Reduced cortical infarct volume, blocked apoptosis, prevented macrophage/microglial infiltration and astrocyte activation</td>
</tr>
<tr>
<td>Iaci et al</td>
<td>rhGGF2</td>
<td>CNS</td>
<td>Chronic stroke (permanent occlusion)</td>
<td>Neuro-restorative effects; functional improvements and improved sensorimotor recovery</td>
</tr>
<tr>
<td>Cai et al</td>
<td>rhNRG-1β</td>
<td>PNS</td>
<td>Sciatic nerve lesion</td>
<td>Improved nerve regeneration across gap</td>
</tr>
<tr>
<td>Krag et al</td>
<td>rhNRG-1β</td>
<td>Skeletal muscle</td>
<td>Muscular dystrophy mouse</td>
<td>Improved muscle function in muscular dystrophy model</td>
</tr>
</tbody>
</table>

CNS indicates central nervous system; NRG indicates neuregulin-1; PNS, peripheral nervous system; rhGGF2, recombinant human glial growth factor 2; rhNRG, recombinant human neuregulin-1.
and ErbB4 on myocardial structure and animal survival, it seems reasonable to speculate that the decreased ErbB receptor expression in late stages of heart failure may contribute to progressive myocardial dysfunction. Interestingly, in patients who had left ventricular unloading with ventricular assist devices, the changes reversed.\textsuperscript{32,34} Whether this contributes to the reversal of gene expression changes and improved myocardial function that is seen in some patients remains to be determined.

**Endogenous NRG-1/ErbB Signaling Protects the Heart From Ischemic Injury**

Ischemia/reperfusion injury is a potent activator of NRG-1/ErbB signaling in the intact heart.\textsuperscript{27} Selective disruption of endothelial NRG-1 expression impairs recovery of cardiac contractile function after an ischemic insult.\textsuperscript{35} Similar results are observed if the heart is perfused with a small-molecule inhibitor of ErbB2 tyrosine kinase activity.\textsuperscript{28} NRG-1 preconditioning has cardioprotective effects against ischemia/reperfusion injury via a PI3K/Akt-dependent mechanism.\textsuperscript{77} Whether the activation of NRG-1 by ischemia/reperfusion also participates in the well-known phenomenon of ischemic preconditioning is unknown.

**NRG as a Potential Therapy for Myocardial Injury and Heart Failure**

Multiple in vivo studies have established the therapeutic potential for recombinant human (rh) NRG-1\textsubscript{β} (Table 2). An EGF-domain fragment of rhNRG-1\textsubscript{β} was used in a series of small and large animal models of systolic heart failure to demonstrate an effect on heart function and survival.\textsuperscript{42} In rats with systolic dysfunction induced by surgical myocardial infarction, intravenous doses of the rhNRG-1\textsubscript{β} fragment administered daily for 5 days improved cardiac function. Similar beneficial effects on cardiac function were reported in models of anthracycline and virally induced cardiac injury in mice. In large animals with rapid-pacing–induced heart failure, the rhNRG-1\textsubscript{β} fragment similarly improved cardiac contractility and relaxation.\textsuperscript{32,78}

A second form of rhNRG-1\textsubscript{β} undergoing examination is a kringle and Ig domain–containing version of NRG-1\textsubscript{β} known as glial growth factor 2 (GGF2). The Ig domain is expressed in all myocardial NRG-1 gene products and localizes ligand through interaction with matrix molecules. In vitro assays of myocyte response to recombinant NRG-1\textsubscript{β}, the EGF domain–only fragment of NRG-1\textsubscript{β} has equal potency in activating intracellular signaling compared with the larger GGF2, but is shorter-acting.\textsuperscript{79} GGF2 treatment of rats or swine with myocardial infarction–induced myocardial dysfunction is associated with improved systolic function and reduced progressive remodeling.\textsuperscript{80,81}

As discussed, there are many effects of NRG-1\textsubscript{β} on the biology of heart and vascular cells that could be contributing to the beneficial effects of NRG-1\textsubscript{β} on cardiac function. Protection of myocytes from stimuli that lead to cell death, together with effects on sarcomere gene expression and stability, may lead to improved cardiac function through repair of dysfunctional cardiac myocytes. NRG-1\textsubscript{β} seems to induce myocyte proliferation in rodents.\textsuperscript{31} At this point, it is unclear whether NRG-1\textsubscript{β}-induced cardiac myocyte proliferation contributes to the improvement in cardiac function observed. In swine, GGF2 treatment is associated with markedly improved mitochondrial structure by electron microscopy.\textsuperscript{82} This is consistent with reports of improved mitochondrial function in animals treated with NRG-1\textsubscript{β}.\textsuperscript{83} Collectively, this work suggests

### Table 2. Studies of Effects of Recombinant NRG-1 on Heart Failure (Animal Studies)

<table>
<thead>
<tr>
<th>Author</th>
<th>Formulation of NRG-1</th>
<th>Heart Failure Model</th>
<th>Outcome</th>
</tr>
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<tbody>
<tr>
<td>Liu et al\textsuperscript{42}</td>
<td>rhNRG-1 EGF domain only</td>
<td>Ischemic/post-MI (rats)</td>
<td>Attenuated dysfunction and improvements in cardiac performance. Increased capillary abundance in the fibrotic peri-infarct area (angiogenesis)</td>
</tr>
<tr>
<td>Bersell et al\textsuperscript{31}</td>
<td>rhNRG-1 (+Ig domain)</td>
<td>Ischemic/post-MI (mice)</td>
<td>Improved echocardiographic parameter and survival</td>
</tr>
<tr>
<td>Gu et al\textsuperscript{39}</td>
<td>rhNRG-1 EGF domain only</td>
<td>Post-MI–induced heart failure (mice)</td>
<td>Improved LV function and cardiac contractility and relaxation</td>
</tr>
<tr>
<td>Guo et al\textsuperscript{40}</td>
<td>rhNRG-1 EGF domain only</td>
<td>Post-MI–induced heart failure (rats)</td>
<td>Improved LV remodeling and cardiac function, reducing mitochondrial dysfunction, myocardocyte apoptosis, and oxidative stress</td>
</tr>
<tr>
<td>Bian et al\textsuperscript{30}</td>
<td>rhGGF2</td>
<td>Doxorubicin-induced heart failure (mice)</td>
<td>Improved survival and cardiac function in doxorubicin-induced heart failure</td>
</tr>
<tr>
<td>Hill et al\textsuperscript{40}</td>
<td>rhGGF2</td>
<td>Post-MI–induced heart failure (rats)</td>
<td>Improved LV contractile function, corrected altered gene expression.</td>
</tr>
<tr>
<td>Kasasbeh et al\textsuperscript{81}</td>
<td>rhGGF2</td>
<td>Post-MI–induced heart failure (swine)</td>
<td>Improved LV contractile function, no effect on diastolic function or inotropic reserve</td>
</tr>
</tbody>
</table>

EGF indicates epidermal growth factor; LV, left ventricular; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; MI, myocardial infarction; NRG indicates neuregulin-1; rhNRG, recombinant human neuregulin-1; rhGGF2, recombinant human glial growth factor 2.
that the effects of NRG-1β on cardiac contractile function are pleiotropic.

The promising preclinical animal studies have led to ongoing clinical trials examining whether these rhNRG-1βs have similar effects in humans. A phase II, double-blinded, placebo-controlled study to evaluate the efficacy and safety of recombinant EGF-domain rhNRG-1β in patients with chronic heart failure has been completed. Patients with New York Heart Association functional class II or III systolic heart failure received placebo or EGF-domain rhNRG-1β (0.3, 0.6, or 1.2 μg/kg per day) by intravenous infusion over a period of hours, daily for 10 days. Three months later, improved cardiac function as measured by magnetic resonance imaging was observed in the intermediate dose group. The acute and chronic responses to EGF-domain rhNRG-1β also have been reported in patients with stable congestive heart failure. Patients receiving an EGF-domain rhNRG-1β infusion daily for 11 days showed an acute increase in cardiac output that was accompanied by a vasodilator effect. At 12 weeks after treatment, there was a 12% increase in ventricular systolic function from baseline. The authors concluded that treatment with EGF-domain rhNRG-1β in patients with stable congestive heart failure on optimal medical therapy produced favorable acute and chronic hemodynamic effects. Larger trials are ongoing in China and Australia and are registered to begin in the United States (ZS-01-210). The phase I studies in patients with heart failure using a full-length glycosylated GGF2 are ongoing (GGF2-1101-1, www.ClinicalTrials.gov).

It seems unlikely that the improvement in cardiac function in response to recombinant NRG treatment is because of a direct inotropic effect. In fact, it seems that recombinant NRG-1β suppresses the increased fractional shortening induced by β-adrenergic receptor activation. There are effects of NRG-1β on calcium transients that may improve myocyte relaxation. Acute inodilator effects of rhNRG-1β have been observed, although it seems more likely that any long-term beneficial effects of rhNRG-1β are occurring through improvements in myocardial structure and function. Preclinical data support exploration of rhNRG-1β in other areas of cardiovascular medicine (Figure 4). In addition to reducing the magnitude of ischemic heart and brain injury, there are effects of NRG-1β on vascular ErbB receptor expression after carotid balloon injury. Intravenous NRG-1β reduces neointimal hyperplasia after injury and prevents mitogen-stimulated vascular smooth muscle cell proliferation and migration. In human samples, NRG-1β expression was amplified in atherosclerotic lesions in coronary artery disease and inhibits atherogenesis via suppression of macrophage foam cell formation.

As rhNRG-1β moves forward as a potential therapeutic for heart failure, it will be important to consider the potential for adverse effects of systemic rhNRG-1β. One obvious concern is its potential to stimulate tumor growth. However, oncological studies have demonstrated that NRG-1 is both a tumor growth factor and a tumor suppressor, preventing generalization of what will be the effects of rhNRG-1β on tumor growth. Also concerning is that NRG/ErbB signaling has been implicated in the pathogenesis of schizophrenia, and behavioral effects of augmented NRG activity in the brain have been observed in mice. Efforts to localize delivery to specific target tissues and minimizing duration of exposure may help in this regard.

**Role of NRG as a Potential Biomarker**

As discussed, NRG-1β is activated in skeletal muscle by exercise and in the heart by injury and by changes in hemodynamic load. These findings have prompted measurements of NRG-1β in the serum and plasma. In healthy subjects, NRG-1β levels correlate with fitness. In contrast, levels correlate with disease prognosis in the subjects with chronic heart failure. In this latter study, higher-serum NRG-1β levels were particularly associated with poorer prognosis in ischemic heart disease. In patients with stable coronary disease without ischemia or heart failure, NRG-1β levels were inversely associated with coronary arterial disease severity. Interestingly, NRG-1β levels were higher in patients with stress-induced ischemia. These results are consistent with the concept that...
myocardial NRG-1β is activated in response to ischemia as observed in the isolated heart.27 The possibility that blood NRG-1β levels may have use as a clinical indicator of health and cardiovascular disease warrants further investigation.

Conclusion

Although a great deal has been learned about the role of NRG-1 and ErbB receptors in the cardiovascular system, there are many important unanswered questions. Knowledge in this field has been stimulated by effects of ErbB2-targeted therapies on the heart, demonstrating the continued importance of interdisciplinary team approaches. Our understanding of what will succeed. Furthermore, the potential for adverse effects of recombinant NRGs or small-molecule activators of ErbB receptor signaling in the cardiovascular system as a possible therapy for heart failure is ongoing. However, the mechanisms by which ErbB activation improves cardiac function are not clear and warrant further investigation. The question of whether cardiac myocyte proliferation in response to NRG treatment plays any role in the improved cardiac function with NRG treatment remains to be answered. There seem to be many determinants of myocardial ErbB receptor expression and, therefore, NRG responsiveness. Understanding these will enhance the likelihood that NRG development as a therapy for heart failure will succeed. Furthermore, the potential for adverse effects of recombinant ErbB activators should prompt continued interdisciplinary team approaches. Our understanding of what regulates the expression and activity of endogenous myocardial NRGs is rudimentary at best. Perhaps, with further investigation, we will discover strategies to augment these signals in a clinically useful way.

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