**Name of journal: World Journal of Hypertension**

**ESPS Manuscript NO: 14315**

**Columns: REVIEW**

**Progress in neuregulin/ErbB signaling and chronic heart failure**

Yin HK *et al*. Role of NRG-1 in heart failure

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**Conflict-of-interest:** The authors declare that they have no conflicts of interest related to this work.

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**Received:** September 28, 2014

**Peer-review started:** September 29, 2014

**First decision:** January 20, 2015

**Revised:** March 10, 2015

**Accepted:** April 16, 2015

**Article in press:**

**Published online:**

**Abstract**

Heart failure is one of the leading causes of death today. It is a complex clinical syndrome in which the heart has a reduced contraction ability and decreased viable myocytes. Novel approaches to the clinical management of heart failure have been achieved through an understanding of the molecular pathways necessary for normal heart development. Neuregulin-1 (NRG-1) has emerged as a potential therapeutic target based on the fact that mice null for *NRG-1* or receptors mediating its activity, *ErbB2* and *ErbB4*, are embryonic lethal and exhibit severe cardiac defects. Preclinical studies performed with animal models of heart failure demonstrate that treatment with NRG-1 significantly improves heart function and survival. Clinical data further support NRG-1 as a promising drug candidate for the treatment of cardiac dysfunction in patients. Recent studies have revealed the mechanism underlying the therapeutic effects of NRG-1/ErbB signaling in the treatment of heart failure. Through activation of upstream signaling molecules such as phosphoinositide 3-kinase, mitogen-activated protein kinase, and focal adhesion kinase, NRG-1/ErbB pathway activation results in increased cMLCK expression and enhanced intracellular calcium cycling. The former is a regulator of the contractile machinery, and the latter triggers cell contraction and relaxation. In addition, NRG-1/ErbB signaling also influences energy metabolism and induces epigenetic modification in cardiac myocytes in a way that more closely resembles healthy heart. These observations reveal potentially new treatment options for heart failure.

**Key words**: ErbB; Epigenetic modification; Heart failure; Metabolism; Neuregulin-1

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**Core tip:** Neuregulin (NRG)-1/ErbB signaling plays a critical role in the development of the heart and the maintenance of cardiac function. In both pre-clinical and clinical studies, NRG-1 has demonstrated efficacy as a therapeutic agent for the treatment of heart failure. In model animals and clinical trials, short-term treatment with recombinant NRG-1 protein results in a long-term beneficial effect. Here, the mechanisms underlying the therapeutic effects of NRG-1 during heart failure are reviewed. The results indicate that NRG-1 induces a cardiac reverse remodeling process through the initiation of changes in both cell metabolism and epigenetic modification.

Yin HK, Li XY, Jiang ZG, Zhou MD. Progress in neuregulin/ErbB signaling and chronic heart failure.*World J Hypertens* 2015; In press

**INTRODUCTION**

The neuregulins (NRGs) are a group of growth factors that regulate multiple cellular processes, including proliferation, apoptosis, adhesion, differentiation, metabolism, and epigenetic modification, through the activation of ErbB receptors and downstream signaling pathways. Increasing evidence demonstrates that NRG-1/ErbB signaling plays a critical role in the development of the heart and the maintenance of cardiac function. In both pre-clinical and clinical studies, NRG-1 has demonstrated efficacy as a therapeutic agent for the treatment of heart failure. This review will focus on the underlying mechanisms and recent achievements in the treatment of heart failure with NRG therapy.

**NRG FAMILY AND THEIR RECEPTORS**

NRGs are ligands for receptor tyrosine kinases of the ErbB family. In mammals, NRGs are a family of homologous proteins encoded by four genes, *NRG1*, *NRG2*, *NRG3*, and *NRG4*. NRG-1 is the most abundant family member expressed in the cardiovascular system and the only NRG currently known to play a role in the development and function of the heart[[1-4](#_ENREF_1)].

Six NRG-1 isoforms generated by alternative splicing have been identified. All NRG-1 isoforms contain an epidermal growth factor (EGF)-like domain, which is critical for function. Proteolytic cleavage at the C-terminal end of the domain results in the release of a secreted, bioactive form of NRG-1[[5](#_ENREF_5),[6](#_ENREF_6)]. Due to alternative splicing, the EGF-like domain of NRG-1 differs at the C-terminal end. An α- or β- variant is generated, and *in vitro* studies have demonstrated that NRG-1 isoforms are 10–100-fold more biologically active than NRG-1 isoforms[[3](#_ENREF_3),[7-9](#_ENREF_7)].

NRG-1 is a growth factor that elicits function through interaction with the ErbB family of tyrosine kinase receptors and is regulated by stress[[10](#_ENREF_10),[11](#_ENREF_11)]. The ErbB family contains four members: ErbB1, ErbB2, ErbB3, and ErbB4. ErbB1, also known as EGF receptor, does not bind NRG-1[[2](#_ENREF_2)]. ErbB2 does not directly bind any ligands, but functions as the heterodimeric partner of the other three ErbB family members[[12](#_ENREF_12)]. NRG-1 binds to ErbB3 and ErbB4, which results in the formation of ErbB2/ErbB3 and ErbB2/ErbB4 heterodimers and leads to the phosphorylation of cytoplasmic receptor tyrosine residues. Multiple intracellular signal transduction cascades, such as phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (Erk) 1/2, and focal adhesion kinase (FAK), are induced and stimulate cell proliferation, differentiation, and survival in many tissues including the heart[[13-15](#_ENREF_13)].

**NRG-1/ERBB SIGNALING IN CARDIAC DEVELOPMENT AND HEART FAILURE**

The importance of NRG-1 in heart development was demonstrated in *Nrg1*-knockout mice. The *Nrg1* knockout was embryonic lethal, with the animals exhibiting cardiac developmental defects, such as the absence of ventricular trabeculation and insufficient myocyte differentiation[[16](#_ENREF_16),[17](#_ENREF_17)]. Such results indicate that NRG-1 activity during cardiac development is not functionally redundant among family members[[18-20](#_ENREF_18)]. The fact that NRG-2 and NRG-3 are expressed in the central nervous system and NRG-4 is expressed in pancreas and skeletal muscle further underscores the essential role for NRG-1 in cardiac development. Proteolytic cleavage is critical for the function of NRG-1, *Adam17*-knockout mice died at birth[[21](#_ENREF_21)]. Interestingly, a deletion mutation in the cytoplasmic tail of NRG-1 is resistant to proteolysis and cannot activate ErbB receptors, suggesting that the intracellular domain is essential for the proteolytic processing of NRG-1 proteins[[22](#_ENREF_22)]. Mice with disrupted *ErbB2* or *ErbB4* were also embryonic lethal before day 11, mirroring the phenotype of the *Nrg1*-knockout mice[[23](#_ENREF_23),[24](#_ENREF_24)]. These findings implicate an essential role in cardiac development for NRG-1/ErbB2/ErbB4 signaling. *ErbB3*, however, is only expressed in mesenchymal cells of the endocardial cushion of the fetal heart. *ErbB3*-knockout mice were embryonic lethal at day 13.5 with defects in the endocardial cushion; however, the trabeculae had developed normally[[24-26](#_ENREF_24)].

A function for NRG-1/ErbB2/ErbB4 signaling has also been confirmed in the adult heart[[27](#_ENREF_27)]. Expression of *NRG-1* is found in the microvascular endothelial cells in the adult heart, but not in the large coronary arteries or in the aorta[[10](#_ENREF_10)]. *ErbB2* and *ErbB4* are expressed in adult cardiomyocytes, while *ErbB3* is only expressed in fetal myocytes[[27](#_ENREF_27)]. However, in one recent study, ErbB3 expression was detected in the adult myocardium, although its function in adult heart still remains to be determined[[28](#_ENREF_28)]. Mice with a cardiac-specific knockout of *ErbB2* were phenotypically normal at birth, but spontaneously developed dilated cardiomyopathy at eight weeks of life. These animals were furthermore unable to survive pressure overload induced by aortic binding, and cardiac hypertrophy markers, skeletal α-actin and atrial natriuretic peptide, also significantly increased during the progression of heart failure[[29](#_ENREF_29)]. The same result was observed in transgenic mice with a cardiomyocyte-specific null mutation in *ErbB2*[[30](#_ENREF_30)]. In addition, the *ErbB4* conditional-knockout mice developed dilated cardiomyopathy with delayed conduction and impaired contractility by the third month after birth[[31](#_ENREF_31)]. Based on these results, ErbB2/ErbB4 appears to be critical also for the maintenance of normal function of the adult heart.

In clinical trials, breast cancer patients treated with trastuzumab (a humanized monoclonal ErbB2-targeted antibody) were found to have an increased risk for symptomatic heart failure and cardiac dysfunction[[32](#_ENREF_32),[33](#_ENREF_33)]. This finding provided strong evidence for the critical role of ErbB2 in the adult human heart. In adult rat ventricular myocytes, treatment with NRG-1 resulted in activation of Erk1/2 and Akt, and significantly inhibited anthracycline-induced myofilament disarray. In contrast, simultaneous treatment of myocytes with anti-ErbB2 and doxorubicin led to more severe myofibrillar disarray than doxorubicin alone[[34](#_ENREF_34)]. In the stress-induced rat model, administration of NRG-1 also led to significant improvement in the prevention of cardiac dilatation[[35](#_ENREF_35)]. These results implicate a role for NRG-1/ErbB signaling in the maintenance of adult cardiac myocyte function and structure. Interestingly, *NRG1* mRNA levels were found to be increased in chronic heart failure patients, while the expression of *ERBB2* and *ERBB4* was reduced in a potential feedback mechanism[[6](#_ENREF_6),[36](#_ENREF_36)], indicating a possible role for NRG-1/ErbB signaling during heart failure.

**POSSIBLE MECHANISMS MEDIATING NRG-1/ERBB SIGNALING IN ADULT HEART**

Based on *in vitro* and *in vivo* studies of cardiac myocytes, NRG-1/ErbB signaling regulates a number of cellular processes by activating signaling pathways such as PI3K/Akt, MAPK-Erk1/2, and FAK[[15](#_ENREF_15),[27](#_ENREF_27),[34](#_ENREF_34),[37](#_ENREF_37)]. These canonical signaling cascades have been extensively reviewed elsewhere and will be addressed very briefly in this review[[1](#_ENREF_1),[38](#_ENREF_38),[39](#_ENREF_39)]. In addition, recent studies indicate that NRG-1 functions as an effector molecule regulating energy metabolism[[7](#_ENREF_7)] and epigenetic modification in cardiomyocytes[[40](#_ENREF_40)]. A working model for NRG-1/ErbB signaling in heart is summarized in Figure 1.

**CANONICAL SIGNALING PATHWAYS MEDIATING NRG-1/ERBB ACTIVITY**

The PI3K/Akt pathway has been well studied in cell proliferation, growth, and apoptosis. In cardiac myocytes, activated Akt signaling inhibits apoptosis[[41](#_ENREF_41),[42](#_ENREF_42)] and protects cardiomyocytes from apoptosis induced by serum starvation[[27](#_ENREF_27)], cardiotoxic anthracycline[[43](#_ENREF_43)], as well as -adrenergic receptor activation[[44](#_ENREF_44),[45](#_ENREF_45)]. This protective effect is dependent on the downstream activation of members of the Bcl-2 family, which typically block apoptosis[[45](#_ENREF_45),[46](#_ENREF_46)]. Interestingly, NRG-1 shows a biphasic dose effect on p70S6K (a downstream protein kinase in the Akt/mTOR pathway) phosphorylation, as higher NRG-1 concentration leads to a decreased response[[13](#_ENREF_13)]. In addition, Akt also promotes glucose uptake as well as activates endothelial nitric oxide synthase, which may contribute to cell survival under metabolic stress[[7](#_ENREF_7),[47](#_ENREF_47)].

 In adult cardiac myocytes, NRG-1 stimulates the Erk1/2 pathway, which leads to expression of genes associated with cardiac hypertrophy[[13](#_ENREF_13)] as well as myofilament organization[[34](#_ENREF_34),[37](#_ENREF_37)]. Erk1/2 activation is mediated by Grb2, Grb7, and Shc, which are downstream targets of ErbB2 and thus, also play a role in cardiac hypertrophy[[48-51](#_ENREF_48)].

FAK signaling is involved in the formation of focal adhesion complexes as well as the restoration of sarcomeres in cardiac myocytes[[52](#_ENREF_52),[53](#_ENREF_53)], and contributes to the growth and survival of myocytes[[54](#_ENREF_54),[55](#_ENREF_55)]. In addition, cardiomyocyte FAK conditional knockout in mice was embryonic lethal, and embryos exhibited a phenotype similar to the ErbB2 or ErbB4 cardiac-specific knockout mice[[56](#_ENREF_56),[57](#_ENREF_57)]. These results provide evidence for a role of FAK in cardiac development.

 Recent studies have identified cardiac myosin light chain kinase (cMLCK) as a downstream target of NRG-1/ErbB signaling in cardiomyocytes[[58](#_ENREF_58)]. As a cardiac specific kinase[[59](#_ENREF_59)], cMLCK is capable of activating myosin light chain[[60](#_ENREF_60)], resulting in sarcomere organization[[61](#_ENREF_61)]. Ventricular myocyte hypertrophy was found in cMLCK-deficient mice with histologic evidence of necrosis and fibrosis[[62](#_ENREF_62)]. In our previous study, adenovirus-mediated gene delivery of cMLCK significantly improved cardiac function of post-myocardial infarction (MI) rats, and RNA interference of cMLCK reduced the beneficial effect of recombinant human NRG-1, rhNRG-1 (Ser177–Glu237 of the EGF-like domain of human NRG-12a developed by scientists at Zensun Company; Shanghai, China), on sarcomere organization[[58](#_ENREF_58)]. Interestingly, although the cMLCK-knockout mice had attenuated MLC phosphorylation and decreased fraction shortening, NRG-1 infusion still improved cardiac performance, indicating that the beneficial effect of NRG-1 on heart function is not completely mediated by cMLCK[[63](#_ENREF_63)].

 Disruption of calcium homeostasis also occurs during the development of heart failure[[64](#_ENREF_64),[65](#_ENREF_65)]. Sarcoplasmic reticulum Ca2+-ATPase 2a (SERCA2a) is a Ca2+-ATPase that regulates calcium uptake and contributes to cardiomyocyte relaxation[[66](#_ENREF_66),[67](#_ENREF_67)]. SERCA2a activity is negatively regulated by phospholamban, a target of protein phosphatase 1[[68](#_ENREF_68),[69](#_ENREF_69)]. It has been reported that rhNRG-1 enhances the intracellular calcium cycle in post-MI rats through the suppression of protein phosphatase 1 expression, which results in the improved SERCA2a activity[[58](#_ENREF_58)]. The first clinical trial of gene therapy using adeno-associated virus (AAV) in the treatment of heart failure was performed in the United States. Both the safety and efficacy of SERCA2a delivery by gene transfer through a recombinant AAV1/SERCA2a were evaluated in patients with advanced heart failure[[70](#_ENREF_70),[71](#_ENREF_71)]. A further 250 patients are currently being enrolled in a phase 2b trial for intracoronary administration of AAV1/SERCA2a[[72](#_ENREF_72)].

**EPIGENETIC MODIFICATION**

Chronic heart failure is considered to be a remodeling process affected by multiple environmental factors, and too complex to be addressed by single pathway interventions[[73](#_ENREF_73)]. NRG-1 treatment results in long-lasting benefits in animal models and human studies, indicating that NRG-1 at least partially stimulates cardiac reverse remodeling, as evidenced by a switch to fetal gene expression, rather than merely preventing cardiac dysfunction[[35](#_ENREF_35)]. DNA methylation is one epigenetic mechanism known to directly regulate the expression of genes by altering the binding of transcription factors to DNA recognition elements[[74](#_ENREF_74)], and dynamic DNA methylation/demethylation has been observed *in vivo*[[75](#_ENREF_75)].

 Epigenetic modification has been linked to cardiac hypertrophy and heart failure[[76](#_ENREF_76)]. For example, class II histone deacetylases (HDACs) suppress cardiac hypertrophy, partially through inhibition of the activity of myocyte enhancer factor 2[[77](#_ENREF_77)]. In contrast, inhibition of HDAC activity results in increased cell size[[78](#_ENREF_78)] and sarcomere disorganization in cultured cardiac myocytes[[79](#_ENREF_79)]. Furthermore, the activity of histone acetyltransferase cofactors, such as cyclic AMP response element-binding protein (CREB)-binding protein and p300, is required in phenylephrine-induced cardiomyocyte hypertrophy[[80](#_ENREF_80)]. In a model for congestive heart failure, the Dahl salt-sensitive rat[[81](#_ENREF_81)], H3K4 and H3K9 were identified as two primary histone modification sites that were markedly altered in cardiac myocytes during the development of the disease. High-throughput analysis performed by chromatin immunoprecipitation of H3K4 or H3K9 on DNA prepared from human heart also revealed global epigenetic changes in cardiac myocytes, and changes occurred in multiple signaling pathways previously associated with the progression of heart failure[[82](#_ENREF_82)].

In cultured rat Schwann cells, NRG-1 dose-dependently activated the transcription factor CREB, a protein with endogenous histone acetyltransferase activity[[83](#_ENREF_83)]. In cultured muscle cells, NRG-1 activated mitogen and stress-activated kinase 1 and 2 and phosphorylated histone H3 in an Erk-dependent manner, resulting in chromatin remodeling[[40](#_ENREF_40)]. Such results implicate a role for NRG-1 in epigenetic modification as well as provide a possible molecular mechanism.

Expression profiles of mRNA from NRG-1 treated and untreated cardiomyocytes have also been compared[[58](#_ENREF_58),[84](#_ENREF_84),[85](#_ENREF_85)]. In our previous study, post-MI rats were infused with rhNRG-1, and the total RNA extracted from the non-infarcted area of the left ventricle was analyzed on GeneChip arrays (Affymetrix, Santa Clara, CA, United States). The results demonstrated that improvement in cardiac function was accompanied by an increase in expression of several epigenetic-related genes[[58](#_ENREF_58)](Table 1).

The global epigenetic changes observed in our study reveal epigenetic modification as an important molecular mechanism underlying changes in cardiac myocytes induced by rhNRG-1 treatment. How these epigenetic changes are triggered by rhNRG-1 requires further investigation. Epigenetic modification also plays an important role in the development of cardiac dysfunction as well as hypertrophy, so that characterization of the epigenetic changes that occur will also help to improve our understanding of the molecular basis of heart failure.

**CELL METABOLISM**

Normal cardiac function relies on the maintenance of energetic homeostasis to a large degree. The cardiac myocyte is a highly oxidative cell type that utilizes mitochondrial respiration to generate most of its energy. In newborn heart, about half of the ATP production is derived from glycolysis[[86](#_ENREF_86)]. After birth, fatty acid oxidation is significantly increased and accompanied by a parallel decrease in glycolytic rates[[87](#_ENREF_87)]. The energy generated by mitochondrial oxidation is primarily derived from the fatty acid -oxidation pathway, and in healthy heart, -oxidation of fatty acids provides more than two thirds of cardiac energy[[88](#_ENREF_88)].

Metabolic abnormalities are clearly involved in the development of heart failure; however, controversy remains concerning the specific alterations in cardiac metabolism and the underlying mechanisms. In late-stage heart failure induced in dogs through pacing-overdrive, fatty acid oxidation-related enzymes were found to be downregulated, while the rate of glucose oxidation dramatically increased[[89](#_ENREF_89),[90](#_ENREF_90)]. Analysis of 13C nuclear magnetic resonance demonstrated that fatty acid oxidation was suppressed in hypertrophic, compensated heart, whereas lactate and glucose oxidation were unaffected[[91](#_ENREF_91)]. In contrast, pressure overload-induced hypertrophy in a rat model exhibited a significant increase only in glucose oxidation[[92](#_ENREF_92)]. This phenomenon was confirmed in a second rat model, in which supra-renal aortic constriction was used to induce hypertrophy; glycolytic capacity was modestly elevated but no significant decline in fatty acid oxidation occurred in the hypertrophic heart[[93](#_ENREF_93)]. These conflicting observations highlight the complexity of energy metabolism in the failing heart.

Emerging evidence indicates that the shift in substrate preference from fatty acids towards glucose in cardiac myocytes can improve heart function and slow the progression of heart failure[[94](#_ENREF_94)], possibly due to the fact that fatty acids waste more ATPs in cardiac metabolism[[95](#_ENREF_95),[96](#_ENREF_96)]. Furthermore, in advanced or end-stage heart failure, the levels of long- and medium-chain acyl-CoA dehydrogenases were dramatically downregulated, resulting in the suppression of fatty acid oxidation[[88](#_ENREF_88)]. Thus, a switch to carbohydrate metabolism appears to improve heart function in the short term, whereas fatty acid oxidation benefits long-term cardiac reverse remodeling.

In a different NRG-1 study, freshly isolated adult rat cardiomyocytes were treated with recombinant human NRG-1 (Neomarkers; P.H. Stehelin and Cie; Basel, Switzerland), and expression profiles were generated with cDNA arrays[[84](#_ENREF_84)]. Expression reprogramming of several cellular processes was revealed, such as improved redox regulation, enhanced utilization of carbohydrates, and increased fatty acid -oxidation[[84](#_ENREF_84)]. In our experiments, rats with sustained MI were intravenously infused with rhNRG-1, and microarray analysis was performed. Expression profiling revealed alterations in a number of genes, including carnitine palmitoyltransferase-1, a key enzyme responsible for the mitochondrial entry of fatty acids[[97](#_ENREF_97)]. A series of fatty acid metabolism enzymes were also upregulated in myocardium[[58](#_ENREF_58)] (Table 2). Our microarray data therefore support a model where cardiac fatty acid -oxidation is increased during rhNRG-1 treatment, and this model is consistent with the observation that rhNRG-1 plays a role in reverse remodeling. However, the causality between energy metabolism and NRG-1-induced reverse remodeling is still an unanswered question, and thus whether a shift in metabolism is the cause or consequence of remodeling requires further investigation.

**PRECLINICAL STUDIES WITH NRG-1 FOR THE TREATMENT OF HEART FAILURE**

Multiple isoforms of NRG-1 in humans are generated as a result of alternative splicing. Preclinical *in vivo* studies have demonstrated that several of the isoforms are capable of improving heart function by reducing hypertension[[47](#_ENREF_47)], improving cardiomyocyte proliferation[[27](#_ENREF_27)], inhibiting apoptosis[[43](#_ENREF_43)], and enhancing angiogenesis[[98](#_ENREF_98)] and Ca2+ handling[[99](#_ENREF_99)]. rhNRG-1 was used in a series of animal models to evaluate its effect on heart function[[35](#_ENREF_35)]. Intravenous administration of rhNRG-1 significantly improved cardiac function and survival in a rat model of heart failure induced by the ligation of left anterior descending coronary artery. In the heart failure model induced by chronic pacing, rhNRG-1 treatment improved the left ventricular end diastolic and systolic pressures, as well as cardiac contractility and relaxation. In addition, a second recombinant form of NRG-1 (recombinant human glial growth factor 2, rhGGF2) also prevented cardiac dysfunction and improved survival in doxorubicin-induced heart failure in the mouse[[100](#_ENREF_100)].

An engineered bivalent human NRG-1 (generated through the synthetic linkage of two NRG-1 moieties) protected against acute doxorubicin-induced cardiomyopathy without proneoplastic effects[[101](#_ENREF_101)]. In another study, administration of recombinant human NRG-1 (Novartis Pharmaceuticals, Basel, Switzerland) significantly improved heart function and reversed cardiac remodeling of diabetic cardiomyopathy in rats with chronic heart failure[[102](#_ENREF_102)]. In addition, rhGGF2 treatment improved residual left ventricular function and normalized a number of myocardial genes altered by MI in rats[[85](#_ENREF_85)].

**CLINICAL STUDIES OF NRG-1/ERBB IN HEART FAILURE**

During the past 30 years, many drugs have been developed for the treatment of heart failure, including -blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and brain natriuretic peptide. Despite the fact that these therapies have improved clinical outcomes significantly, heart failure has become the major cause of cardiovascular death[[103](#_ENREF_103)]. Therefore, the development of new treatments for heart failure continues to be necessary.

Multiple *in vitro* and *in vivo* studies have confirmed the beneficial effects of NRG-1 on cardiac function[[27](#_ENREF_27),[98](#_ENREF_98),[99](#_ENREF_99)], thus rendering NRG-1 a promising drug candidate for the treatment of heart failure. To date, two different isoforms of NRG-1 have been tested in human clinical trials. Since 2004, phase 1 and phase 2 trials in China, Australia, and the United States have confirmed that rhNRG-1 is safe and well tolerated in both chronic heart failure patients and healthy controls. In a phase 2, randomized, double-blind, multicenter, placebo-controlled study, 44 patients with New York Heart Association functional class II or III stable chronic heart failure were randomly assigned to four groups and treated with placebo or rhNRG-1 (0.3 μg/kg per day, 0.6 μg/kg per day, or 1.2 μg/kg per day) through a ten-hour intravenous infusion per day for ten consecutive days. At day 30, patients treated with rhNRG-1 exhibited significantly increased left ventricular ejection fraction (LVEF%), as well as reduced end-diastolic and end-systolic volumes, which continued to decrease at day 90 and were accompanied by a sustained increase in LVEF%, indicating a long-term effect for rhNRG-1 in cardiac reverse remodeling[[104](#_ENREF_104)]. In another clinical trial, 15 patients with stable chronic heart failure received a daily infusion of rhNRG-1 for 11 days. Improved hemodynamic effects were observed, and the increase in LVEF% was sustained for 12 wk[[105](#_ENREF_105)]. A phase 3 trial designed to measure the safety and efficacy of rhNRG-1 in a larger cohort of chronic heart failure patients is currently ongoing in China.

Another NRG-1 isoform utilized in clinical trials is GGF2 (also known as NRG-13). In a phase 1, single-infusion, dose-escalation study, a single dose of rhGGF2 was well tolerated up to 0.75 mg/kg, whereas higher doses were associated with serious adverse events[[106](#_ENREF_106)]. Patients with symptomatic heart failure receiving a single dose of rhGGF2 exhibited increased left ventricular function over 28 days compared to placebo[[107](#_ENREF_107)]. A phase 1b study designed to evaluate the effect of rhGGF2 single intravenous infusion on midazolam pharmacokinetics is ongoing (registration at www.clinicaltials.gov, NCT01944683).

A complicating issue is the fact that *ERBB2* has a well-described role as an oncogene, particularly in the development of breast cancers[[108](#_ENREF_108),[109](#_ENREF_109)]. Although recent publications support the idea that *NRG1* functions instead as a tumor-suppressor gene[[110](#_ENREF_110)], NRG-1 treatment for cardiac therapy raises a concern for a potential increased risk of cancer. However, ErbB2-associated cancer is often NRG-independent, and furthermore *NRG1* is often silenced by methylation in breast cancers[[111](#_ENREF_111)]. In addition, chromosome translocation breakpoints targeting *NRG1* on 8p12 have been found in breast and pancreas cancer cell lines[[112](#_ENREF_112),[113](#_ENREF_113)]. Finally, our previous clinical experience demonstrated that the incidence of cancer of any type in> 1000 subjects treated with rhNRG-1was no different than in patients treated with placebo. Together, these findings indicate that there is a low risk for the development of cancer during NRG-1 treatment.

**CONCLUSION**

A number of experimental results from both clinical studies and animal models have demonstrated the importance of NRG-1/ErbB signaling in adult heart function. Expression profiling has firmly established that in addition to canonical ErbB2 downstream pathways, energy metabolism and epigenetic modification also play roles in NRG-1-mediated reverse remodeling of heart failure. Additional studies, however, are still necessary to elucidate the precise molecular mechanisms utilized. Finally, a recombinant human NRG-1 peptide has demonstrated significant potential as a novel drug candidate for chronic heart failure in preclinical and clinical studies. Further studies illuminating mechanisms mediating NRG-1/ErbB signaling will therefore help to facilitate the development of novel strategies for the treatment of chronic heart failure and to better understand the function of NRG-1 in cardiac physiology.

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**P-Reviewer:** **Ananiev J,** Lin GM, Kato TS **S-Editor:** Qi Y

**L-Editor: E-Editor:**

**Figure 1** **Role of neuregulin-1/ErbB signaling in heart.** Neuregulin (NRG)-1 treatment affects various signaling pathways as well as leads to changes in cell metabolism and epigenetic modification that more closely resemble normal heart function. Akt: Protein kinase B; cMLK: Cardiac myosin light-chain kinase; eNOS: Endothelial nitric oxide synthase; Erk: Extracellular signal-regulated kinase; FAK: Focal adhesion kinase; MLC: Myosin light chain; PLB: Phospholamban; PP1: Protein phosphatase 1; SERCA2a: Sarcoplasmic reticulum Ca2+-ATPase 2a.

**Table 1** **Changes in mRNA levels of chromosome remodeling and histone modification genes in rat cardiomyocytes treated with rhNRG-1β**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Fold increase****(rhNRG-β/vehicle)** | **Biologic process** | **Ref.** |
| Embryonic ectoderm development (*Eed*) | 1.56 | Genetic imprinting, histone methylation | [[114](#_ENREF_114)] |
| SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (*Smarca4*) | 1.48 | Nucleosome disassembly, methylation-dependent chromatin silencing, ATP-dependent chromatin remodeling | [[115](#_ENREF_115)] |
| Jumonji domain containing 6 (*Jmjd6*) | 1.61 | Histone H3-R2 demethylation, histone H4-R3 demethylation, histone lysyl 5-hydroxylation | [[116](#_ENREF_116), [117](#_ENREF_117)] |
| Histone cluster 1, H4b (*Hist1h4b*) | 1.49 | Nucleosome assembly | [[118](#_ENREF_118)] |
| CSRP2 binding protein (*Csrp2bp*) | 2.00 | Histone acetylation | [[119](#_ENREF_119)] |
| H2A histone family, member Z (*H2afz*) | 1.63 | Nucleosome assembly | [[120](#_ENREF_120)] |
| MYST histone acetyltransferase (monocytic leukemia) 3 (*Myst3*) | 1.48 | Chromatin modification, histone acetylation | [[121](#_ENREF_121)] |
| Nuclear receptor coactivator 3 (*Ncoa3*) | 2.33 | Chromatin modification, histone acetylation | [[122](#_ENREF_122)] |
| Nucleophosmin (nucleolar phosphoprotein B23, numatrin) (*Nmp1*) | 1.80 | Nucleosome assembly | [[123](#_ENREF_123)] |

**Table 2** **Changes in mRNA levels of fatty acid metabolism enzyme genes in rat cardiomyocytes treated with rhNRG-1β**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Fold increase****(rhNRG-1β */*vehicle)** | **Function** | **Ref.** |
| Carnitine palmitoyltransferase Ib, muscle (*Cpt1b*) | 1.83 | Rate-limiting enzyme which imports fatty acid for mitochondrial oxidation | [[124](#_ENREF_124)] |
| Acyl-CoA synthetase, long-chain family 4 (*Acsl4*) | 2.31 | Promotes fatty acid uptake | [[125](#_ENREF_125)] |
| 2,4-dienoyl CoA reductase, mitochondrial (*Decr1*) | 2.04 | Catalyzes the rate-limiting step that prepares polyunsaturated fatty acids to be utilized as substrates for β-oxidation | [[126](#_ENREF_126)] |
| Hydroxyacyl-CoA dehydrogenase (*Hadhb*) | 1.59 | β-subunit of the mitochondrial trifunctional protein, catalyzes the last three steps of mitochondrial β-oxidation of long-chain fatty acids | [[127](#_ENREF_127), [128](#_ENREF_128)] |
| Transketolase (*Tkt*) | 1.97 | Necessary for the production of NADPH, especially in tissues actively engaged in biosyntheses, such as fatty acid synthesis | [[129](#_ENREF_129)] |
| Acetyl-CoA acetyltransferase 1 (*Acat1*) | 1.89 | Enzyme participates in ten metabolic pathways including fatty acid metabolism | [[130](#_ENREF_130)] |
| Hydroxysteroid (17-beta) dehydrogenase 4 (*Hsd17b4*) | 1.64 | Enzyme involved in peroxisomal fatty acid β-oxidation | [[131](#_ENREF_131)] |
| Dodecenoyl-coenzyme A delta isomerase (*Dci*) | 1.66 | Mitochondrial fatty acid oxidation enzyme | [[132](#_ENREF_132)] |
| Protein kinase, AMP-activated, β 1 non-catalyticsubunit (*Prkab*1) | 1.98 | Regulatory subunit of the AMP-activated protein kinase, involved in regulating *de novo* biosynthesis of fatty acid and cholesterol | [[133](#_ENREF_133)] |