World Journal of Hypertension

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5494/wjh.v5.i2.63

*World J Hypertens* 2015 May 23; 5(2): 63-73
ISSN 2220-3168 (online)


REVIEW

# Progress in neuregulin/ErbB signaling and chronic heart failure

Hong-Kun Yin, Xin-Yan Li, Zheng-Gang Jiang, Ming-Dong Zhou

Hong-Kun Yin, Xin-Yan Li, Zheng-Gang Jiang, Ming-Dong Zhou, Zensun (Shanghai) Sci and Tech Co., Ltd., Zhangjiang Hi-tech Park, Shanghai 201203, China
Author contributions: Yin HK wrote the manuscript; Li XY, Jiang ZG and Zhou MD revised the manuscript.
Conflict-of-interest: The authors declare that they have no conflicts of interest related to this work.

Correspondence to: Ming-Dong Zhou, PhD, Zensun (Shanghai) Sci and Tech Co., Ltd., Zhangjiang Hi-tech Park, No. 68 Ju Li Road, Shanghai 201203, China. mdzhou@zensun.com
Telephone: +86-21-50802627
Fax: +86-21-50802621
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: April 18, 2015
Published online: May 23, 2015

## 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



**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; 5(2): 63-73 Available from: URL: http://www.wjgnet.com/2220-3168/full/v5/i2/63.htm DOI: http://dx.doi.org/10.5494/wjh.v5.i2.63

## 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].

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[5,6]. 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-1β isoforms are 10-100-fold more biologically active than NRG-1α isoforms[3,7-9].

NRG-1 is a growth factor that elicits function through interaction with the ErbB family of tyrosine kinase receptors and is regulated by stress[10,11]. The ErbB family contains four members: ErbB1, ErbB2, ErbB3, and ErbB4. ErbB1, also known as EGF receptor, does not bind NRG-1[2]. ErbB2 does not directly bind any ligands, but functions as the heterodimeric partner of the other three ErbB family members[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].

## 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[16,17]. Such results indicate that NRG-1 activity during cardiac development is not functionally redundant among family members[18-20]. 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]. 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]. Mice with disrupted *ErbB2* or *ErbB4* were also embryonic lethal before day 11, mirroring the phenotype of the *Nrg1*-knockout mice[23,24]. 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].

A function for NRG-1/ErbB2/ErbB4 signaling has also been confirmed in the adult heart[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]. *ErbB2* and *ErbB4* are expressed in adult cardiomyocytes, while *ErbB3* is only expressed in fetal myocytes[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]. 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]. The same result was observed in transgenic mice with a cardiomyocyte-specific null mutation in *ErbB2*[30]. In addition, the *ErbB4* conditional-knockout mice developed dilated cardiomyopathy with delayed conduction and impaired contractility by the third month after birth[31]. Based on these results, ErbB2/ErbB4 appears to be critical also for the maintenance of normal

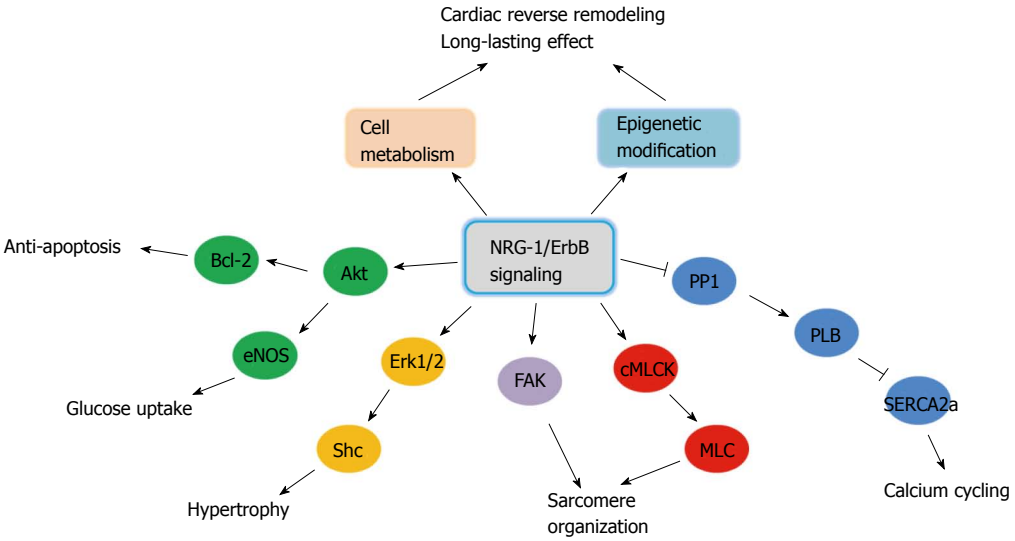


**Figure 1 Role of neuregulin-1/ErbB signaling in heart.** Neuregulin (NRG)-1 treatment affects various signaling pathways as well as leads to changes in cell metabolism and epigenetic modification that more closely resemble normal heart function. Akt: Protein kinase B; cMLK: Cardiac myosin light-chain 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 $Ca^{2+}$-ATPase 2a.

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[32,33]. This finding provided strong evidence for the critical role of ErbB2 in the adult human heart. In adult rat ventricular myocytes, treatment with NRG-1β 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]. In the stress-induced rat model, administration of NRG-1β also led to significant improvement in the prevention of cardiac dilatation[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[6,36], 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[15,27,34,37]. These canonical signaling cascades have been extensively reviewed elsewhere and will be addressed very briefly in this review[1,38,39]. In addition, recent studies indicate that NRG-1 functions as an effector molecule regulating energy metabolism[7] and epigenetic modification in cardiomyocytes[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[41,42] and protects cardiomyocytes from apoptosis induced by serum starvation[27], cardiotoxic anthracycline[43], as well as β-adrenergic receptor activation[44,45]. This protective effect is dependent on the downstream activation of members of the Bcl-2 family, which typically block apoptosis[45,46]. 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]. In addition, Akt also promotes glucose uptake as well as activates endothelial nitric oxide synthase, which may contribute to cell survival under metabolic stress[7,47].

In adult cardiac myocytes, NRG-1 stimulates the Erk1/2 pathway, which leads to expression of genes associated with cardiac hypertrophy[13] as well as myofilament organization[34,37]. 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].

FAK signaling is involved in the formation of focal adhesion complexes as well as the restoration of sarcomeres in cardiac myocytes[52,53], and contributes to the growth and survival of myocytes[54,55]. 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[56,57]. 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]. As a cardiac specific kinase[59], cMLCK is capable of activating myosin light chain[60], resulting in sarcomere organization[61]. Ventricular myocyte hypertrophy was found in cMLCK-deficient mice with histologic evidence of necrosis and fibrosis[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-1β (Ser177-Glu237 of the EGF-like domain of human NRG-1β2a developed by scientists at Zensun Company; Shanghai, China), on sarcomere organization[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].

Disruption of calcium homeostasis also occurs during the development of heart failure[64,65]. Sarcoplasmic reticulum $Ca^{2+}$-ATPase 2a (SERCA2a) is a $Ca^{2+}$-ATPase that regulates calcium uptake and contributes to cardiomyocyte relaxation[66,67]. SERCA2a activity is negatively regulated by phospholamban, a target of protein phosphatase 1[68,69]. It has been reported that rhNRG-1β 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]. 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[70,71]. A further 250 patients are currently being enrolled in a phase 2b trial for intracoronary administration of AAV1/SERCA2a[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]. 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]. 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], and dynamic DNA methylation/demethylation has been observed *in vivo*[75].

Epigenetic modification has been linked to cardiac hypertrophy and heart failure[76]. For example, class Ⅱ histone deacetylases (HDACs) suppress cardiac hypertrophy, partially through inhibition of the activity of myocyte enhancer factor 2[77]. In contrast, inhibition of HDAC activity results in increased cell size[78] and sarcomere disorganization in cultured cardiac myocytes[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]. In a model for congestive heart failure, the Dahl salt-sensitive rat[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].

In cultured rat Schwann cells, NRG-1β dose-dependently activated the transcription factor CREB, a protein with endogenous histone acetyltransferase activity[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]. 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[58,84,85]. In our previous study, post-MI rats were infused with rhNRG-1β, 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] (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-1β treatment. How these epigenetic changes are triggered by rhNRG-1β 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]. After birth, fatty acid oxidation is significantly increased and accompanied

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

| Gene | Fold increase (rhNRG-1β/vehicle) | Biologic process | Ref. |
|---|---|---|---|
| Embryonic ectoderm development (*Eed*) | 1.56 | Genetic imprinting, histone methylation | [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] |
| Jumonji domain containing 6 (*Jmjd6*) | 1.61 | Histone H3-R2 demethylation, histone H4-R3 demethylation, histone lysyl 5-hydroxylation | [116,117] |
| Histone cluster 1, H4b (*Hist1h4b*) | 1.49 | Nucleosome assembly | [118] |
| CSRP2 binding protein (*Csrp2bp*) | 2 | Histone acetylation | [119] |
| H2A histone family, member Z (*H2afz*) | 1.63 | Nucleosome assembly | [120] |
| MYST histone acetyltransferase (monocytic leukemia) 3 (*Myst3*) | 1.48 | Chromatin modification, histone acetylation | [121] |
| Nuclear receptor coactivator 3 (*Ncoa3*) | 2.33 | Chromatin modification, histone acetylation | [122] |
| Nucleophosmin (nucleolar phosphoprotein B23, numatrin) (*Nmp1*) | 1.8 | Nucleosome assembly | [123] |

NRG: Neuregulin.

by a parallel decrease in glycolytic rates[87]. The energy generated by mitochondrial oxidation is primarily derived from the fatty acid β-oxidation pathway, and in healthy heart, β-oxidation of fatty acids provides more than two thirds of cardiac energy[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[89,90]. Analysis of $^{13}C$ nuclear magnetic resonance demonstrated that fatty acid oxidation was suppressed in hypertrophic, compensated heart, whereas lactate and glucose oxidation were unaffected[91]. In contrast, pressure overload-induced hypertrophy in a rat model exhibited a significant increase only in glucose oxidation[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]. 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], possibly due to the fact that fatty acids waste more ATPs in cardiac metabolism[95,96]. 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]. 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-1β (Neomarkers; P.H. Stehelin and Cie; Basel, Switzerland), and expression profiles were generated with cDNA arrays[84]. Expression reprogramming of several cellular processes was revealed, such as improved redox regulation, enhanced utilization of carbohydrates, and increased fatty acid β-oxidation[84]. In our experiments, rats with sustained MI were intravenously infused with rhNRG-1β, 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]. A series of fatty acid metabolism enzymes were also upregulated in myocardium[58] (Table 2). Our microarray data therefore support a model where cardiac fatty acid β-oxidation is increased during rhNRG-1β treatment, and this model is consistent with the observation that rhNRG-1β 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], improving cardiomyocyte proliferation[27], inhibiting apoptosis[43], and enhancing angiogenesis[98] and $Ca^{2+}$ handling[99]. rhNRG-1β was used in a series of animal models to evaluate its effect on heart function[35]. Intravenous administration of rhNRG-1β significantly improved cardiac function and survival in

**Table 2 Changes in mRNA levels of fatty acid metabolism enzyme genes in rat cardiomyocytes treated with rhneuregulin-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] |
| Acyl-CoA synthetase, long-chain family 4 (*Acsl4*) | 2.31 | Promotes fatty acid uptake | [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] |
| 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 | [127,128] |
| Transketolase (*Tkt*) | 1.97 | Necessary for the production of NADPH, especially in tissues actively engaged in biosyntheses, such as fatty acid synthesis | [129] |
| Acetyl-CoA acetyltransferase 1 (*Acat1*) | 1.89 | Enzyme participates in ten metabolic pathways including fatty acid metabolism | [130] |
| Hydroxysteroid (17-beta) dehydrogenase 4 (*Hsd17b4*) | 1.64 | Enzyme involved in peroxisomal fatty acid β-oxidation | [131] |
| Dodecenoyl-coenzyme A delta isomerase (*Dci*) | 1.66 | Mitochondrial fatty acid oxidation enzyme | [132] |
| Protein kinase, AMP-activated, β1 non-catalytic subunit (*Prkab1*) | 1.98 | Regulatory subunit of the AMP-activated protein kinase, involved in regulating de novo biosynthesis of fatty acid and cholesterol | [133] |

NRG: Neuregulin.

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-1β 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].

An engineered bivalent human NRG-1β (generated through the synthetic linkage of two NRG-1β moieties) protected against acute doxorubicin-induced cardiomyopathy without proneoplastic effects[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]. In addition, rhGGF2 treatment improved residual left ventricular function and normalized a number of myocardial genes altered by MI in rats[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 β-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]. 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[27,98,99], 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-1β 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-1β (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-1β 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-1β in cardiac reverse remodeling[104]. In another clinical trial, 15 patients with stable chronic heart failure received a daily infusion of rhNRG-1β for 11 d. Improved hemodynamic effects were observed, and the increase in LVEF% was sustained for 12 wk[105]. A phase 3 trial designed to measure the safety and efficacy of rhNRG-1β 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-1β3). 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]. Patients with symptomatic heart failure receiving a single dose of rhGGF2 exhibited increased left

ventricular function over 28 d compared to placebo[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[108,109]. Although recent publications support the idea that *NRG1* functions instead as a tumor-suppressor gene[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]. In addition, chromosome translocation breakpoints targeting *NRG1* on 8p12 have been found in breast and pancreas cancer cell lines[112,113]. Finally, our previous clinical experience demonstrated that the incidence of cancer of any type in > 1000 subjects treated with rhNRG-1β 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.

## REFERENCES


1 **Pentassuglia L**, Sawyer DB. The role of Neuregulin-1beta/ErbB signaling in the heart. *Exp Cell Res* 2009; **315**: 627-637 [PMID: 18801360 DOI: 10.1016/j.yexcr.2008.08.015]

2 **Britsch S**. The neuregulin-I/ErbB signaling system in development and disease. *Adv Anat Embryol Cell Biol* 2007; **190**: 1-65 [PMID: 17432114]

3 **Falls DL**. Neuregulins: functions, forms, and signaling strategies. *Exp Cell Res* 2003; **284**: 14-30 [PMID: 12648463 DOI: 10.1016/S0014-4827(02)00102-7]

4 **Fuller SJ**, Sivarajah K, Sugden PH. ErbB receptors, their ligands, and the consequences of their activation and inhibition in the myocardium. *J Mol Cell Cardiol* 2008; **44**: 831-854 [PMID: 18430438 DOI: 10.1016/j.yjmcc.2008.02.278]

5 **Parodi EM**, Kuhn B. Signalling between microvascular endothelium and cardiomyocytes through neuregulin. *Cardiovasc Res* 2014; **102**: 194-204 [PMID: 24477642 DOI: 10.1093/cvr/cvu021]

6 **Lemmens K**, Doggen K, De Keulenaer GW. Role of neuregulin-1/ErbB signaling in cardiovascular physiology and disease: implications for therapy of heart failure. *Circulation* 2007; **116**: 954-960 [PMID: 17709650 DOI: 10.1161/circulationaha.107.690487]

7 **Cote GM**, Miller TA, Lebrasseur NK, Kuramochi Y, Sawyer DB. Neuregulin-1alpha and beta isoform expression in cardiac microvascular endothelial cells and function in cardiac myocytes in vitro. *Exp Cell Res* 2005; **311**: 135-146 [PMID: 16185687 DOI: 10.1016/j.yexcr.2005.08.017]

8 **Lu HS**, Chang D, Philo JS, Zhang K, Narhi LO, Liu N, Zhang M, Sun J, Wen J, Yanagihara D. Studies on the structure and function of glycosylated and nonglycosylated neu differentiation factors. Similarities and differences of the alpha and beta isoforms. *J Biol Chem* 1995; **270**: 4784-4791 [PMID: 7876251 DOI: 10.1074/jbc.270.9.4784]

9 **Hobbs SS**, Coffing SL, Le AT, Cameron EM, Williams EE, Andrew M, Blommel EN, Hammer RP, Chang H, Riese DJ. Neuregulin isoforms exhibit distinct patterns of ErbB family receptor activation. *Oncogene* 2002; **21**: 8442-8452 [PMID: 12466964 DOI: 10.1038/sj.onc.1205960]

10 **Lemmens K**, Segers VF, Demolder M, De Keulenaer GW. Role of neuregulin-1/ErbB2 signaling in endothelium-cardiomyocyte cross-talk. *J Biol Chem* 2006; **281**: 19469-19477 [PMID: 16698793 DOI: 10.1074/jbc.M600399200]

11 **Yarden Y**, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001; **2**: 127-137 [PMID: 11252954 DOI: 10.1038/35052073]

12 **Bublil EM**, Yarden Y. The EGF receptor family: spearheading a merger of signaling and therapeutics. *Curr Opin Cell Biol* 2007; **19**: 124-134 [PMID: 17314037 DOI: 10.1016/j.ceb.2007.02.008]

13 **Baliga RR**, Pimental DR, Zhao YY, Simmons WW, Marchionni MA, Sawyer DB, Kelly RA. NRG-1-induced cardiomyocyte hypertrophy. Role of PI-3-kinase, p70(S6K), and MEK-MAPK-RSK. *Am J Physiol* 1999; **277**: H2026-H2037 [PMID: 10564160]

14 **Muthuswamy SK**, Gilman M, Brugge JS. Controlled dimerization of ErbB receptors provides evidence for differential signaling by homo- and heterodimers. *Mol Cell Biol* 1999; **19**: 6845-6857 [PMID: 10490623]

15 **Kuramochi Y**, Guo X, Sawyer DB. Neuregulin activates erbB2-dependent src/FAK signaling and cytoskeletal remodeling in isolated adult rat cardiac myocytes. *J Mol Cell Cardiol* 2006; **41**: 228-235 [PMID: 16769082 DOI: 10.1016/j.yjmcc.2006.04.007]

16 **Kramer R**, Bucay N, Kane DJ, Martin LE, Tarpley JE, Theill LE. Neuregulins with an Ig-like domain are essential for mouse myocardial and neuronal development. *Proc Natl Acad Sci USA* 1996; **93**: 4833-4838 [PMID: 8643489]

17 **Meyer D**, Birchmeier C. Multiple essential functions of neuregulin in development. *Nature* 1995; **378**: 386-390 [PMID: 7477375 DOI: 10.1038/378386a0]

18 **Ring HZ**, Chang H, Guilbot A, Brice A, LeGuern E, Francke U. The human neuregulin-2 (NRG2) gene: cloning, mapping and evaluation as a candidate for the autosomal recessive form of Charcot-Marie-Tooth disease linked to 5q. *Hum Genet* 1999; **104**: 326-332 [PMID: 10369162 DOI: 10.1007/s004390050961]

19 **Hayes NV**, Newsam RJ, Baines AJ, Gullick WJ. Characterization of the cell membrane-associated products of the Neuregulin 4 gene. *Oncogene* 2008; **27**: 715-720 [PMID: 17684490 DOI: 10.1038/sj.onc.1210689]

20 **Harari D**, Tzahar E, Romano J, Shelly M, Pierce JH, Andrews GC, Yarden Y. Neuregulin-4: a novel growth factor that acts through the ErbB-4 receptor tyrosine kinase. *Oncogene* 1999; **18**: 2681-2689 [PMID: 10348342 DOI: 10.1038/sj.onc.1202631]

21 **Shi W**, Chen H, Sun J, Buckley S, Zhao J, Anderson KD, Williams RG, Warburton D. TACE is required for fetal murine cardiac development and modeling. *Dev Biol* 2003; **261**: 371-380 [PMID: 14499647 DOI: 10.1016/S0012-1606(03)00315-4]

22 **Liu X**, Hwang H, Cao L, Buckland M, Cunningham A, Chen J, Chien KR, Graham RM, Zhou M. Domain-specific gene disruption reveals critical regulation of neuregulin signaling by its cytoplasmic tail. *Proc Natl Acad Sci USA* 1998; **95**: 13024-13029 [PMID:

9789034 DOI: 10.1073/pnas.95.22.13024]
23 **Lee KF**, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* 1995; **378**: 394-398 [PMID: 7477377 DOI: 10.1038/378394a0]
24 **Gassmann M**, Casagranda F, Orioli D, Simon H, Lai C, Klein R, Lemke G. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* 1995; **378**: 390-394 [PMID: 7477376 DOI: 10.1038/378390a0]
25 **Erickson SL**, O'Shea KS, Ghaboosi N, Loverro L, Frantz G, Bauer M, Lu LH, Moore MW. ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2-and heregulin-deficient mice. *Development* 1997; **124**: 4999-5011 [PMID: 9362461]
26 **Camenisch TD**, Schroeder JA, Bradley J, Klewer SE, McDonald JA. Heart-valve mesenchyme formation is dependent on hyaluronan-augmented activation of ErbB2-ErbB3 receptors. *Nat Med* 2002; **8**: 850-855 [PMID: 12134143 DOI: 10.1038/nm742]
27 **Zhao YY**, Sawyer DR, Baliga RR, Opel DJ, Han X, Marchionni MA, Kelly RA. Neuregulins promote survival and growth of cardiac myocytes. Persistence of ErbB2 and ErbB4 expression in neonatal and adult ventricular myocytes. *J Biol Chem* 1998; **273**: 10261-10269 [PMID: 9553078]
28 **Campreciós G**, Lorita J, Pardina E, Peinado-Onsurbe J, Soley M, Ramírez I. Expression, localization, and regulation of the neuregulin receptor ErbB3 in mouse heart. *J Cell Physiol* 2011; **226**: 450-455 [PMID: 20672328 DOI: 10.1002/jcp.22354]
29 **Crone SA**, Zhao YY, Fan L, Gu Y, Minamisawa S, Liu Y, Peterson KL, Chen J, Kahn R, Condorelli G, Ross J, Chien KR, Lee KF. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat Med* 2002; **8**: 459-465 [PMID: 11984589 DOI: 10.1038/nm0502-459]
30 **Ozcelik C**, Erdmann B, Pilz B, Wettschureck N, Britsch S, Hübner N, Chien KR, Birchmeier C, Garratt AN. Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. *Proc Natl Acad Sci USA* 2002; **99**: 8880-8885 [PMID: 12072561 DOI: 10.1073/pnas.122249299]
31 **García-Rivello H**, Taranda J, Said M, Cabeza-Meckert P, Vila-Petroff M, Scaglione J, Ghio S, Chen J, Lai C, Laguens RP, Lloyd KC, Hertig CM. Dilated cardiomyopathy in Erb-b4-deficient ventricular muscle. *Am J Physiol Heart Circ Physiol* 2005; **289**: H1153-H1160 [PMID: 15863464 DOI: 10.1152/ajpheart.00048.2005]
32 **Slamon DJ**, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; **344**: 783-792 [PMID: 11248153 DOI: 10.1056/nejm200103153441101]
33 **Jones LW**, Haykowsky M, Peddle CJ, Joy AA, Pituskin EN, Tkachuk LM, Courneya KS, Slamon DJ, Mackey JR. Cardiovascular risk profile of patients with HER2/neu-positive breast cancer treated with anthracycline-taxane-containing adjuvant chemotherapy and/or trastuzumab. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1026-1031 [PMID: 17507633 DOI: 10.1158/1055-9965.epi-06-0870]
34 **Sawyer DB**, Zuppinger C, Miller TA, Eppenberger HM, Suter TM. Modulation of anthracycline-induced myofibrillar disarray in rat ventricular myocytes by neuregulin-1beta and anti-erbB2: potential mechanism for trastuzumab-induced cardiotoxicity. *Circulation* 2002; **105**: 1551-1554 [PMID: 11927521]
35 **Liu X**, Gu X, Li Z, Li X, Li H, Chang J, Chen P, Jin J, Xi B, Chen D, Lai D, Graham RM, Zhou M. Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. *J Am Coll Cardiol* 2006; **48**: 1438-1447 [PMID: 17010808 DOI: 10.1016/j.jacc.2006.05.057]
36 **Rohrbach S**, Niemann B, Silber RE, Holtz J. Neuregulin receptors erbB2 and erbB4 in failing human myocardium -- depressed expression and attenuated activation. *Basic Res Cardiol* 2005; **100**: 240-249 [PMID: 15685397 DOI: 10.1007/s00395-005-0514-4]
37 **Pentassuglia L**, Timolati F, Seifriz F, Abudukadier K, Suter TM, Zuppinger C. Inhibition of ErbB2/neuregulin signaling augments paclitaxel-induced cardiotoxicity in adult ventricular myocytes. *Exp Cell Res* 2007; **313**: 1588-1601 [PMID: 17400210 DOI: 10.1016/j.yexcr.2007.02.007]
38 **Odiete O**, Hill MF, Sawyer DB. Neuregulin in cardiovascular development and disease. *Circ Res* 2012; **111**: 1376-1385 [PMID: 23104879 DOI: 10.1161/circresaha.112.267286]
39 **Jiang Z**, Zhou M. Neuregulin signaling and heart failure. *Curr Heart Fail Rep* 2010; **7**: 42-47 [PMID: 20425496 DOI: 10.1007/s11897-010-0003-y]
40 **Basu U**, Gyrd-Hansen M, Baby SM, Lozynska O, Krag TO, Jensen CJ, Frödin M, Khurana TS. Heregulin-induced epigenetic regulation of the utrophin-A promoter. *FEBS Lett* 2007; **581**: 4153-4158 [PMID: 17692845 DOI: 10.1016/j.febslet.2007.07.021]
41 **Matsui T**, Li L, del MonteF Y, Franke TF, Hajjar RJ, Rosenzweig A. Adenoviral gene transfer of activated phosphatidylinositol 3'-kinase and Akt inhibits apoptosis of hypoxic cardiomyocytes in vitro. *Circulation* 1999; **100**: 2373-2379 [PMID: 10587343]
42 **Fujio Y**, Nguyen T, Wencker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. *Circulation* 2000; **101**: 660-667 [PMID: 10673259]
43 **Fukazawa R**, Miller TA, Kuramochi Y, Frantz S, Kim YD, Marchionni MA, Kelly RA, Sawyer DB. Neuregulin-1 protects ventricular myocytes from anthracycline-induced apoptosis via erbB4-dependent activation of PI3-kinase/Akt. *J Mol Cell Cardiol* 2003; **35**: 1473-1479 [PMID: 14654373]
44 **Okoshi K**, Nakayama M, Yan X, Okoshi MP, Schuldt AJ, Marchionni MA, Lorell BH. Neuregulins regulate cardiac parasympathetic activity: muscarinic modulation of beta-adrenergic activity in myocytes from mice with neuregulin-1 gene deletion. *Circulation* 2004; **110**: 713-717 [PMID: 15289373 DOI: 10.1161/01.cir.0000138109.32748.80]
45 **Jamnicki-Abegg M**, Weihrauch D, Pagel PS, Kersten JR, Bosnjak ZJ, Warltier DC, Bienengraeber MW. Isoflurane inhibits cardiac myocyte apoptosis during oxidative and inflammatory stress by activating Akt and enhancing Bcl-2 expression. *Anesthesiology* 2005; **103**: 1006-1014 [PMID: 16249675]
46 **Das S**, Cordis GA, Maulik N, Das DK. Pharmacological preconditioning with resveratrol: role of CREB-dependent Bcl-2 signaling via adenosine A3 receptor activation. *Am J Physiol Heart Circ Physiol* 2005; **288**: H328-H335 [PMID: 15345477 DOI: 10.1152/ajpheart.00453.2004]
47 **Lemmens K**, Fransen P, Sys SU, Brutsaert DL, De Keulenaer GW. Neuregulin-1 induces a negative inotropic effect in cardiac muscle: role of nitric oxide synthase. *Circulation* 2004; **109**: 324-326 [PMID: 14732742 DOI: 10.1161/01.cir.0000114521.88547.5e]
48 **Zhang S**, Weinheimer C, Courtois M, Kovacs A, Zhang CE, Cheng AM, Wang Y, Muslin AJ. The role of the Grb2-p38 MAPK signaling pathway in cardiac hypertrophy and fibrosis. *J Clin Invest* 2003; **111**: 833-841 [PMID: 12639989 DOI: 10.1172/jci16290]
49 **Pero SC**, Shukla GS, Cookson MM, Flemer S, Krag DN. Combination treatment with Grb7 peptide and Doxorubicin or Trastuzumab (Herceptin) results in cooperative cell growth inhibition in breast cancer cells. *Br J Cancer* 2007; **96**: 1520-1525 [PMID: 17426702 DOI: 10.1038/sj.bjc.6603732]
50 **Obreztchikova M**, Elouardighi H, Ho M, Wilson BA, Gertsberg Z, Steinberg SF. Distinct signaling functions for Shc isoforms in the heart. *J Biol Chem* 2006; **281**: 20197-20204 [PMID: 16699171 DOI: 10.1074/jbc.M601859200]
51 **Yoshizumi M**, Tsuchiya K, Kirima K, Kyaw M, Suzaki Y, Tamaki T. Quercetin inhibits Shc- and phosphatidylinositol 3-kinase-mediated c-Jun N-terminal kinase activation by angiotensin II in cultured rat aortic smooth muscle cells. *Mol Pharmacol* 2001; **60**: 656-665 [PMID: 11562426]
52 **Ilić D**, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T. Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. *Nature* 1995; **377**: 539-544 [PMID:

7566154 DOI: 10.1038/377539a0]
53 **Mansour H**, de Tombe PP, Samarel AM, Russell B. Restoration of resting sarcomere length after uniaxial static strain is regulated by protein kinase Cepsilon and focal adhesion kinase. *Circ Res* 2004; **94**: 642-649 [PMID: 14963000 DOI: 10.1161/01.res.0000121101.32286.c8]
54 **Kuppuswamy D**. Importance of integrin signaling in myocyte growth and survival. *Circ Res* 2002; **90**: 1240-1242 [PMID: 12089060]
55 **Pfister R**, Acksteiner C, Baumgarth J, Burst V, Geissler HJ, Margulies KB, Houser S, Bloch W, Flesch M. Loss of beta1D-integrin function in human ischemic cardiomyopathy. *Basic Res Cardiol* 2007; **102**: 257-264 [PMID: 17186162 DOI: 10.1007/s00395-006-0640-1]
56 **Peng X**, Wu X, Druso JE, Wei H, Park AY, Kraus MS, Alcaraz A, Chen J, Chien S, Cerione RA, Guan JL. Cardiac developmental defects and eccentric right ventricular hypertrophy in cardiomyocyte focal adhesion kinase (FAK) conditional knockout mice. *Proc Natl Acad Sci USA* 2008; **105**: 6638-6643 [PMID: 18448675 DOI: 10.1073/pnas.0802319105]
57 **Peng X**, Kraus MS, Wei H, Shen TL, Pariaut R, Alcaraz A, Ji G, Cheng L, Yang Q, Kotlikoff MI, Chen J, Chien K, Gu H, Guan JL. Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. *J Clin Invest* 2006; **116**: 217-227 [PMID: 16374517 DOI: 10.1172/jci24497]
58 **Gu X**, Liu X, Xu D, Li X, Yan M, Qi Y, Yan W, Wang W, Pan J, Xu Y, Xi B, Cheng L, Jia J, Wang K, Ge J, Zhou M. Cardiac functional improvement in rats with myocardial infarction by up-regulating cardiac myosin light chain kinase with neuregulin. *Cardiovasc Res* 2010; **88**: 334-343 [PMID: 20615916 DOI: 10.1093/cvr/cvq223]
59 **Seguchi O**, Takashima S, Yamazaki S, Asakura M, Asano Y, Shintani Y, Wakeno M, Minamino T, Kondo H, Furukawa H, Nakamaru K, Naito A, Takahashi T, Ohtsuka T, Kawakami K, Isomura T, Kitamura S, Tomoike H, Mochizuki N, Kitakaze M. A cardiac myosin light chain kinase regulates sarcomere assembly in the vertebrate heart. *J Clin Invest* 2007; **117**: 2812-2824 [PMID: 17885681 DOI: 10.1172/jci30804]
60 **Warren SA**, Briggs LE, Zeng H, Chuang J, Chang EI, Terada R, Li M, Swanson MS, Lecker SH, Willis MS, Spinale FG, Maupin-Furlowe J, McMullen JR, Moss RL, Kasahara H. Myosin light chain phosphorylation is critical for adaptation to cardiac stress. *Circulation* 2012; **126**: 2575-2588 [PMID: 23095280 DOI: 10.1161/circulationaha.112.116202]
61 **Aoki H**, Sadoshima J, Izumo S. Myosin light chain kinase mediates sarcomere organization during cardiac hypertrophy in vitro. *Nat Med* 2000; **6**: 183-188 [PMID: 10655107 DOI: 10.1038/72287]
62 **Ding P**, Huang J, Battiprolu PK, Hill JA, Kamm KE, Stull JT. Cardiac myosin light chain kinase is necessary for myosin regulatory light chain phosphorylation and cardiac performance in vivo. *J Biol Chem* 2010; **285**: 40819-40829 [PMID: 20943660 DOI: 10.1074/jbc.M110.160499]
63 **Chang AN**, Huang J, Battiprolu PK, Hill JA, Kamm KE, Stull JT. The effects of neuregulin on cardiac Myosin light chain kinase gene-ablated hearts. *PLoS One* 2013; **8**: e66720 [PMID: 23776695 DOI: 10.1371/journal.pone.0066720]
64 **Kubalova Z**, Terentyev D, Viatchenko-Karpinski S, Nishijima Y, Györke I, Terentyeva R, da Cuñha DN, Sridhar A, Feldman DS, Hamlin RL, Carnes CA, Györke S. Abnormal intrastore calcium signaling in chronic heart failure. *Proc Natl Acad Sci USA* 2005; **102**: 14104-14109 [PMID: 16172392 DOI: 10.1073/pnas.0504298102]
65 **Jiang MT**, Lokuta AJ, Farrell EF, Wolff MR, Haworth RA, Valdivia HH. Abnormal Ca2+ release, but normal ryanodine receptors, in canine and human heart failure. *Circ Res* 2002; **91**: 1015-1022 [PMID: 12456487]
66 **Bassani JW**, Yuan W, Bers DM. Fractional SR Ca release is regulated by trigger Ca and SR Ca content in cardiac myocytes. *Am J Physiol* 1995; **268**: C1313-C1319 [PMID: 7762626]
67 **Go LO**, Moschella MC, Watras J, Handa KK, Fyfe BS, Marks AR. Differential regulation of two types of intracellular calcium release channels during end-stage heart failure. *J Clin Invest* 1995; **95**: 888-894 [PMID: 7860772 DOI: 10.1172/jci117739]
68 **MacDougall LK**, Jones LR, Cohen P. Identification of the major protein phosphatases in mammalian cardiac muscle which dephosphorylate phospholamban. *Eur J Biochem* 1991; **196**: 725-734 [PMID: 1849481]
69 **Gupta RC**, Mishra S, Rastogi S, Imai M, Habib O, Sabbah HN. Cardiac SR-coupled PP1 activity and expression are increased and inhibitor 1 protein expression is decreased in failing hearts. *Am J Physiol Heart Circ Physiol* 2003; **285**: H2373-H2381 [PMID: 14613911 DOI: 10.1152/ajpheart.00442.2003]
70 **Hajjar RJ**, Zsebo K, Deckelbaum L, Thompson C, Rudy J, Yaroshinsky A, Ly H, Kawase Y, Wagner K, Borow K, Jaski B, London B, Greenberg B, Pauly DF, Patten R, Starling R, Mancini D, Jessup M. Design of a phase 1/2 trial of intracoronary administration of AAV1/SERCA2a in patients with heart failure. *J Card Fail* 2008; **14**: 355-367 [PMID: 18514926 DOI: 10.1016/j.cardfail.2008.02.005]
71 **Jaski BE**, Jessup ML, Mancini DM, Cappola TP, Pauly DF, Greenberg B, Borow K, Dittrich H, Zsebo KM, Hajjar RJ. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical trial. *J Card Fail* 2009; **15**: 171-181 [PMID: 19327618 DOI: 10.1016/j.cardfail.2009.01.013]
72 **Greenberg B**, Yaroshinsky A, Zsebo KM, Butler J, Felker GM, Voors AA, Rudy JJ, Wagner K, Hajjar RJ. Design of a phase 2b trial of intracoronary administration of AAV1/SERCA2a in patients with advanced heart failure: the CUPID 2 trial (calcium up-regulation by percutaneous administration of gene therapy in cardiac disease phase 2b). *JACC Heart Fail* 2014; **2**: 84-92 [PMID: 24622121 DOI: 10.1016/j.jchf.2013.09.008]
73 **Cohn JN**. Critical review of heart failure: the role of left ventricular remodeling in the therapeutic response. *Clin Cardiol* 1995; **18**: IV4-I12 [PMID: 7489620]
74 **Hendrich B**, Tweedie S. The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends Genet* 2003; **19**: 269-277 [PMID: 12711219 DOI: 10.1016/s0168-9525(03)00080-5]
75 **Métivier R**, Gallais R, Tiffoche C, Le Péron C, Jurkowska RZ, Carmouche RP, Ibberson D, Barath P, Demay F, Reid G, Benes V, Jeltsch A, Gannon F, Salbert G. Cyclical DNA methylation of a transcriptionally active promoter. *Nature* 2008; **452**: 45-50 [PMID: 18322525 DOI: 10.1038/nature06544]
76 **Movassagh M**, Choy MK, Knowles DA, Cordeddu L, Haider S, Down T, Siggens L, Vujic A, Simeoni I, Penkett C, Goddard M, Lio P, Bennett MR, Foo RS. Distinct epigenomic features in end-stage failing human hearts. *Circulation* 2011; **124**: 2411-2422 [PMID: 22025602 DOI: 10.1161/circulationaha.111.040071]
77 **Zhang CL**, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* 2002; **110**: 479-488 [PMID: 12202037]
78 **Iezzi S**, Di Padova M, Serra C, Caretti G, Simone C, Maklan E, Minetti G, Zhao P, Hoffman EP, Puri PL, Sartorelli V. Deacetylase inhibitors increase muscle cell size by promoting myoblast recruitment and fusion through induction of follistatin. *Dev Cell* 2004; **6**: 673-684 [PMID: 15130492]
79 **Antos CL**, McKinsey TA, Dreitz M, Hollingsworth LM, Zhang CL, Schreiber K, Rindt H, Gorczynski RJ, Olson EN. Dose-dependent blockade to cardiomyocyte hypertrophy by histone deacetylase inhibitors. *J Biol Chem* 2003; **278**: 28930-28937 [PMID: 12761226 DOI: 10.1074/jbc.M303113200]
80 **Gusterson RJ**, Jazrawi E, Adcock IM, Latchman DS. The transcriptional co-activators CREB-binding protein (CBP) and p300 play a critical role in cardiac hypertrophy that is dependent on their histone acetyltransferase activity. *J Biol Chem* 2003; **278**: 6838-6847 [PMID: 12477714 DOI: 10.1074/jbc.M211762200]
81 **Rapp JP**, Wang SM, Dene H. A genetic polymorphism in the renin gene of Dahl rats cosegregates with blood pressure. *Science* 1989; **243**: 542-544 [PMID: 2563177 DOI: 10.1126/science.2563177]
82 **Kaneda R**, Takada S, Yamashita Y, Choi YL, Nonaka-Sarukawa M, Soda M, Misawa Y, Isomura T, Shimada K, Mano H. Genome-wide

histone methylation profile for heart failure. *Genes Cells* 2009; **14**: 69-77 [PMID: 19077033 DOI: 10.1111/j.1365-2443.2008.01252.x]

83 **Tabernero A,** Stewart HJS, Jessen KR, Mirsky R.The Neuron-Glia Signal beta Neuregulin Induces Sustained CREB Phosphorylation on Ser-133 in Cultured Rat Schwann Cells. *Mol Cell Neurosci* 1998; **10**: 309-322 [PMID: 9618221 DOI: 10.1006/mcne.1998.0662]

84 **Giraud MN**, Flück M, Zuppinger C, Suter TM. Expressional reprogramming of survival pathways in rat cardiocytes by neuregulin-1beta. *J Appl Physiol* (1985) 2005; **99**: 313-322 [PMID: 16036905 DOI: 10.1152/japplphysiol.00609.2004]

85 **Hill MF**, Patel AV, Murphy A, Smith HM, Galindo CL, Pentassuglia L, Peng X, Lenneman CG, Odiete O, Friedman DB, Kronenberg MW, Zheng S, Zhao Z, Song Y, Harrell FE, Srinivas M, Ganguly A, Iaci J, Parry TJ, Caggiano AO, Sawyer DB. Intravenous glial growth factor 2 (GGF2) isoform of neuregulin-1β improves left ventricular function, gene and protein expression in rats after myocardial infarction. *PLoS One* 2013; **8**: e55741 [PMID: 23437060 DOI: 10.1371/journal.pone.0055741]

86 **Lopaschuk GD**, Spafford MA, Marsh DR. Glycolysis is predominant source of myocardial ATP production immediately after birth. *Am J Physiol* 1991; **261**: H1698-H1705 [PMID: 1750528]

87 **Itoi T**, Lopaschuk GD. The contribution of glycolysis, glucose oxidation, lactate oxidation, and fatty acid oxidation to ATP production in isolated biventricular working hearts from 2-week-old rabbits. *Pediatr Res* 1993; **34**: 735-741 [PMID: 8108185 DOI: 10.1203/00006450-199312000-00008]

88 **Stanley WC**, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 2005; **85**: 1093-1129 [PMID: 15987803 DOI: 10.1152/physrev.00006.2004]

89 **Lei B**, Lionetti V, Young ME, Chandler MP, d'Agostino C, Kang E, Altarejos M, Matsuo K, Hintze TH, Stanley WC, Recchia FA. Paradoxical downregulation of the glucose oxidation pathway despite enhanced flux in severe heart failure. *J Mol Cell Cardiol* 2004; **36**: 567-576 [PMID: 15081316 DOI: 10.1016/j.yjmcc.2004.02.004]

90 **Osorio JC**, Stanley WC, Linke A, Castellari M, Diep QN, Panchal AR, Hintze TH, Lopaschuk GD, Recchia FA. Impaired myocardial fatty acid oxidation and reduced protein expression of retinoid X receptor-alpha in pacing-induced heart failure. *Circulation* 2002; **106**: 606-612 [PMID: 12147544 DOI: 10.1161/01.CIR.0000023531.22727.C1]

91 **Recchia FA**, McConnell PI, Bernstein RD, Vogel TR, Xu X, Hintze TH. Reduced nitric oxide production and altered myocardial metabolism during the decompensation of pacing-induced heart failure in the conscious dog. *Circ Res* 1998; **83**: 969-979 [PMID: 9815144 DOI: 10.1161/01.RES.83.10.969]

92 **Young ME**, Laws FA, Goodwin GW, Taegtmeyer H. Reactivation of peroxisome proliferator-activated receptor alpha is associated with contractile dysfunction in hypertrophied rat heart. *J Biol Chem* 2001; **276**: 44390-44395 [PMID: 11574533 DOI: 10.1074/jbc.M103826200]

93 **Degens H**, de Brouwer KF, Gilde AJ, Lindhout M, Willemsen PH, Janssen BJ, van der Vusse GJ, van Bilsen M. Cardiac fatty acid metabolism is preserved in the compensated hypertrophic rat heart. *Basic Res Cardiol* 2006; **101**: 17-26 [PMID: 16136293 DOI: 10.1007/s00395-005-0549-0]

94 **Stanley WC**, Chandler MP. Energy metabolism in the normal and failing heart: potential for therapeutic interventions. *Heart Fail Rev* 2002; **7**: 115-130 [PMID: 11988636 DOI: 10.1023/A:1015320423577]

95 **Himms-Hagen J**, Harper ME. Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis. *Exp Biol Med* (Maywood) 2001; **226**: 78-84 [PMID: 11446442]

96 **Schrauwen P**, Saris WH, Hesselink MK. An alternative function for human uncoupling protein 3: protection of mitochondria against accumulation of nonesterified fatty acids inside the mitochondrial matrix. *FASEB J* 2001; **15**: 2497-2502 [PMID: 11689475 DOI: 10.1096/fj.01-0400hyp]

97 **Jogl G**, Tong L. Crystal structure of carnitine acetyltransferase and implications for the catalytic mechanism and fatty acid transport. *Cell* 2003; **112**: 113-122 [PMID: 12526798 DOI: 10.1016/S0092-8674(02)01228-X]

98 **Russell KS**, Stern DF, Polverini PJ, Bender JR. Neuregulin activation of ErbB receptors in vascular endothelium leads to angiogenesis. *Am J Physiol* 1999; **277**: H2205-H2211 [PMID: 10600838]

99 **Brero A**, Ramella R, Fitou A, Dati C, Alloatti G, Gallo MP, Levi R. Neuregulin-1beta1 rapidly modulates nitric oxide synthesis and calcium handling in rat cardiomyocytes. *Cardiovasc Res* 2010; **88**: 443-452 [PMID: 20634213 DOI: 10.1093/cvr/cvq238]

100 **Bian Y**, Sun M, Silver M, Ho KK, Marchionni MA, Caggiano AO, Stone JR, Amende I, Hampton TG, Morgan JP, Yan X. Neuregulin-1 attenuated doxorubicin-induced decrease in cardiac troponins. *Am J Physiol Heart Circ Physiol* 2009; **297**: H1974-H1983 [PMID: 19801490 DOI: 10.1152/ajpheart.01010.2008]

101 **Jay SM**, Murthy AC, Hawkins JF, Wortzel JR, Steinhauser ML, Alvarez LM, Gannon J, Macrae CA, Griffith LG, Lee RT. An engineered bivalent neuregulin protects against doxorubicin-induced cardiotoxicity with reduced proneoplastic potential. *Circulation* 2013; **128**: 152-161 [PMID: 23757312 DOI: 10.1161/circulationaha.113.002203]

102 **Li B**, Zheng Z, Wei Y, Wang M, Peng J, Kang T, Huang X, Xiao J, Li Y, Li Z. Therapeutic effects of neuregulin-1 in diabetic cardiomyopathy rats. *Cardiovasc Diabetol* 2011; **10**: 69 [PMID: 21798071 DOI: 10.1186/1475-2840-10-69]

103 **Rosamond W**, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2008; **117**: e25-146 [PMID: 18086926 DOI: 10.1161/circulationaha.107.187998]

104 **Gao R**, Zhang J, Cheng L, Wu X, Dong W, Yang X, Li T, Liu X, Xu Y, Li X, Zhou M. A Phase II, randomized, double-blind, multicenter, based on standard therapy, placebo-controlled study of the efficacy and safety of recombinant human neuregulin-1 in patients with chronic heart failure. *J Am Coll Cardiol* 2010; **55**: 1907-1914 [PMID: 20430261 DOI: 10.1016/j.jacc.2009.12.044]

105 **Jabbour A**, Hayward CS, Keogh AM, Kotlyar E, McCrohon JA, England JF, Amor R, Liu X, Li XY, Zhou MD, Graham RM, Macdonald PS. Parenteral administration of recombinant human neuregulin-1 to patients with stable chronic heart failure produces favourable acute and chronic haemodynamic responses. *Eur J Heart Fail* 2011; **13**: 83-92 [PMID: 20810473 DOI: 10.1093/eurjhf/hfq152]

106 **Lenihan DJ,** Anderson S, Geisberg C, Caggiano A, Eisen A, Brittain E, Muldowney Iii JAS, Mendes L, Sawyer D. Safety and tolerability of glial growth factor 2 in patients with chronic heart failure: a phase I single dose escalation study. *J Am College Cardiol* 2013; **61** (Suppl 10): E707 [DOI: 10.1016/S0735-1097(13)60707-X]

107 **Brittain E,** Muldowney J, Geisberg C, Caggiano A, Eisen A, Anderson S, Sawyer D, Mendes L, Lenihan D. Evaluation of cardiac function in symptomatic heart failure patients in a single infusion, phase 1, dose escalation study of glial growth factor 2. *J Am College Cardiol* 2013; **61** (Suppl 10): E715 [DOI: 10.1016/S0735-1097(13)60715-9]

108 **Yu D**, Hung MC. Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. *Oncogene* 2000; **19**: 6115-6121 [PMID: 11156524 DOI: 10.1038/sj.onc.1203972]

109 **Atlas E**, Cardillo M, Mehmi I, Zahedkargaran H, Tang C, Lupu R. Heregulin is sufficient for the promotion of tumorigenicity and metastasis of breast cancer cells in vivo. *Mol Cancer Res* 2003; **1**: 165-175 [PMID: 12556556]

110 **Alajati A**, Sausgruber N, Aceto N, Duss S, Sarret S, Voshol H, Bonenfant D, Bentires-Alj M. Mammary tumor formation and metastasis evoked by a HER2 splice variant. *Cancer Res* 2013; **73**: 5320-5327 [PMID: 23867476 DOI: 10.1158/0008-5472.can-12-3186]

111 **Chua YL**, Ito Y, Pole JC, Newman S, Chin SF, Stein RC, Ellis IO,

Caldas C, O'Hare MJ, Murrell A, Edwards PA. The NRG1 gene is frequently silenced by methylation in breast cancers and is a strong candidate for the 8p tumour suppressor gene. *Oncogene* 2009; **28**: 4041-4052 [PMID: 19802002 DOI: 10.1038/onc.2009.259]

112 **Adélaïde J**, Huang HE, Murati A, Alsop AE, Orsetti B, Mozziconacci MJ, Popovici C, Ginestier C, Letessier A, Basset C, Courtay-Cahen C, Jacquemier J, Theillet C, Birnbaum D, Edwards PA, Chaffanet M. A recurrent chromosome translocation breakpoint in breast and pancreatic cancer cell lines targets the neuregulin/NRG1 gene. *Genes Chromosomes Cancer* 2003; **37**: 333-345 [PMID: 12800145 DOI: 10.1002/gcc.10218]

113 **Huang HE**, Chin SF, Ginestier C, Bardou VJ, Adélaïde J, Iyer NG, Garcia MJ, Pole JC, Callagy GM, Hewitt SM, Gullick WJ, Jacquemier J, Caldas C, Chaffanet M, Birnbaum D, Edwards PA. A recurrent chromosome breakpoint in breast cancer at the NRG1/neuregulin 1/heregulin gene. *Cancer Res* 2004; **64**: 6840-6844 [PMID: 15466169 DOI: 10.1158/0008-5472.can-04-1762]

114 **Ura H**, Murakami K, Akagi T, Kinoshita K, Yamaguchi S, Masui S, Niwa H, Koide H, Yokota T. Eed/Sox2 regulatory loop controls ES cell self-renewal through histone methylation and acetylation. *EMBO J* 2011; **30**: 2190-2204 [PMID: 21540835 DOI: 10.1038/emboj.2011.126]

115 **Orvis T**, Hepperla A, Walter V, Song S, Simon J, Parker J, Wilkerson MD, Desai N, Major MB, Hayes DN, Davis IJ, Weissman B. BRG1/SMARCA4 inactivation promotes non-small cell lung cancer aggressiveness by altering chromatin organization. *Cancer Res* 2014; **74**: 6486-6498 [PMID: 25115300 DOI: 10.1158/0008-5472.can-14-0061]

116 **Chang B**, Chen Y, Zhao Y, Bruick RK. JMJD6 is a histone arginine demethylase. *Science* 2007; **318**: 444-447 [PMID: 17947579 DOI: 10.1126/science.1145801]

117 **Unoki M**, Masuda A, Dohmae N, Arita K, Yoshimatsu M, Iwai Y, Fukui Y, Ueda K, Hamamoto R, Shirakawa M, Sasaki H, Nakamura Y. Lysyl 5-hydroxylation, a novel histone modification, by Jumonji domain containing 6 (JMJD6). *J Biol Chem* 2013; **288**: 6053-6062 [PMID: 23303181 DOI: 10.1074/jbc.M112.433284]

118 **Albig W**, Kioschis P, Poustka A, Meergans K, Doenecke D. Human histone gene organization: nonregular arrangement within a large cluster. *Genomics* 1997; **40**: 314-322 [PMID: 9119399 DOI: 10.1006/geno.1996.4592]

119 **Weiskirchen R**, Gressner AM. The cysteine- and glycine-rich LIM domain protein CRP2 specifically interacts with a novel human protein (CRP2BP). *Biochem Biophys Res Commun* 2000; **274**: 655-663 [PMID: 10924333 DOI: 10.1006/bbrc.2000.3187]

120 **Hatch CL**, Bonner WM. The human histone H2A.Z gene. Sequence and regulation. *J Biol Chem* 1990; **265**: 15211-15218 [PMID: 1697587]

121 **Dreveny I**, Deeves SE, Fulton J, Yue B, Messmer M, Bhattacharya A, Collins HM, Heery DM. The double PHD finger domain of MOZ/MYST3 induces α-helical structure of the histone H3 tail to facilitate acetylation and methylation sampling and modification. *Nucleic Acids Res* 2014; **42**: 822-835 [PMID: 24150941 DOI: 10.1093/nar/gkt931]

122 **Esteyries S**, Perot C, Adelaide J, Imbert M, Lagarde A, Pautas C, Olschwang S, Birnbaum D, Chaffanet M, Mozziconacci MJ. NCOA3, a new fusion partner for MOZ/MYST3 in M5 acute myeloid leukemia. *Leukemia* 2008; **22**: 663-665 [PMID: 17805331 DOI: 10.1038/sj.leu.2404930]

123 **Lindström MS**. NPM1/B23: A Multifunctional Chaperone in Ribosome Biogenesis and Chromatin Remodeling. *Biochem Res Int* 2011; **2011**: 195209 [PMID: 21152184 DOI: 10.1155/2011/195209]

124 **Yamazaki N**, Yamanaka Y, Hashimoto Y, Shinohara Y, Shima A, Terada H. Structural features of the gene encoding human muscle type carnitine palmitoyltransferase I. *FEBS Lett* 1997; **409**: 401-406 [PMID: 9224698 DOI: 10.1016/S0014-5793(97)00561-9]

125 **Kang MJ**, Fujino T, Sasano H, Minekura H, Yabuki N, Nagura H, Iijima H, Yamamoto TT. A novel arachidonate-preferring acyl-CoA synthetase is present in steroidogenic cells of the rat adrenal, ovary, and testis. *Proc Natl Acad Sci USA* 1997; **94**: 2880-2884 [PMID: 9096315 DOI: 10.1073/pnas.94.7.2880]

126 **Fillgrove KL**, Anderson VE. The mechanism of dienoyl-CoA reduction by 2,4-dienoyl-CoA reductase is stepwise: observation of a dienolate intermediate. *Biochemistry* 2001; **40**: 12412-12421 [PMID: 11591162 DOI: 10.1021/bi0111606]

127 **Eaton S**, Bursby T, Middleton B, Pourfarzam M, Mills K, Johnson AW, Bartlett K. The mitochondrial trifunctional protein: centre of a beta-oxidation metabolon? *Biochem Soc Trans* 2000; **28**: 177-182 [PMID: 10816122]

128 **Mannaerts GP**, Van Veldhoven PP, Casteels M. Peroxisomal lipid degradation via beta- and alpha-oxidation in mammals. *Cell Biochem Biophys* 2000; **32** Spring: 73-87 [PMID: 11330072 DOI: 10.1385/CBB:32:1-3:73]

129 **Nilsson U**, Meshalkina L, Lindqvist Y, Schneider G. Examination of substrate binding in thiamin diphosphate-dependent transketolase by protein crystallography and site-directed mutagenesis. *J Biol Chem* 1997; **272**: 1864-1869 [PMID: 8999873 DOI: 10.1074/jbc.272.3.1864]

130 **Reza JZ**, Doosti M, Salehipour M, Packnejad M, Mojarrad M, Heidari M. Modulation peroxisome proliferators activated receptor alpha (PPAR alpha) and acyl coenzyme A: cholesterol acyltransferase1 (ACAT1) gene expression by fatty acids in foam cell. *Lipids Health Dis* 2009; **8**: 38 [PMID: 19725980 DOI: 10.1186/1476-511x-8-38]

131 **Möller G**, Leenders F, van Grunsven EG, Dolez V, Qualmann B, Kessels MM, Markus M, Krazeisen A, Husen B, Wanders RJ, de Launoit Y, Adamski J. Characterization of the HSD17B4 gene: D-specific multifunctional protein 2/17beta-hydroxysteroid dehydrogenase IV. *J Steroid Biochem Mol Biol* 1999; **69**: 441-446 [PMID: 10419023 DOI: 10.1016/S0960-0760(99)00066-7]

132 **Rasmussen AL**, Diamond DL, McDermott JE, Gao X, Metz TO, Matzke MM, Carter VS, Belisle SE, Korth MJ, Waters KM, Smith RD, Katze MG. Systems virology identifies a mitochondrial fatty acid oxidation enzyme, dodecenoyl coenzyme A delta isomerase, required for hepatitis C virus replication and likely pathogenesis. *J Virol* 2011; **85**: 11646-11654 [PMID: 21917952 DOI: 10.1128/jvi.05605-11]

133 **Stapleton D**, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, House CM, Fernandez CS, Cox T, Witters LA, Kemp BE. Mammalian AMP-activated protein kinase subfamily. *J Biol Chem* 1996; **271**: 611-614 [PMID: 8557660 DOI: 10.1074/jbc.271.2.611]

**P- Reviewer**: Ananiev J, Lin GM, Kato TS **S- Editor**: Qi Y
**L- Editor**: A **E- Editor**: Lu YJ


Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com