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Neuregulin-1 β for the treatment of systolic heart failure

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Keywords

heregulin; ErbB2; ErbB4; cardiac myocyte; glial growth factor 2

Introduction

Systolic heart failure (HF) is a disease characterized by progressive cardiac dysfunction leading to increased morbidity and early mortality. The vast majority of pharmacologic strategies for HF developed over recent decades have focused on inhibiting processes that have been demonstrated to contribute to progression of myocardial remodeling. Unfortunately, drug therapies in HF do not produce “reverse remodeling” or improved systolic function via non-inotropic mechanisms in a uniform or predictable manner.

Biological therapeutics in the form of growth factors and cell therapies have been studied extensively in animal models of heart failure, and have been pursued as potential strategies to induce myocardial repair by design. Recombinant growth hormone (GH) was promising in preclinical models but did not show reproducible benefits in randomized clinical trials of HF [1, 2]. IGF-1 has similarly appeared promising in preclinical studies [3], but has not been pursued vigorously to clinical trials, perhaps due to the concern of IGF-1 mediated effects on other organs including tumor growth promotion.

In the last decade direct and indirect manipulation of cell populations to induce myocardial regeneration have been attempted, with mixed results as far as efficacy and improvement in LV function. Ongoing trials, including those funded by the National Heart Lung and Blood Institute (NHLBI) will help to address whether these strategies can be refined to provide consistent benefit. A consistent finding in preclinical models where cell therapies are being developed suggests that a major mechanism for the beneficial effects of cell-based therapies may be that cells provide a source of paracrine factors that help myocardial repair. Neuregulin-1 β (Nrg-1 β) is one of these paracrine factors with interesting biological properties, and potential therapeutic value.

Neuregulins – paracrine regulators of tissue form and function

Nrgs belong to a family of growth factors which are encoded by one of four known genes (Nrg-1 through -4) and act through receptor tyrosine kinases in the epidermal growth factor

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receptor family (EGFR). Each of the Nrg genes can be expressed as multiple distinct protein isoforms due to alternative splicing of the transcripts [4]. Nrg's actions are mediated through a set of ErbB tyrosine kinase receptors (ErbB2, ErbB2, ErbB4) which stimulate cellular proliferation, differentiation, and survival of cells in many tissues including the heart. (Table 1).

Since their initial identification Nrgs have been named according to their function in various tissues (e.g. skeletal muscle: ARIA – Acetylcholine Receptor Inducing Activity; Her2/neu expressing tumor cells: NDF – Neu Differentiation Factor; central nervous system: GGF - Glial Growth Factor). Nrgs are also named according to the splice variants, distinguishing alpha or beta variants of the EGF-like domain, as well as indicating the linker sequence before the transmembrane domain (e.g. the type II neuregulin GGF2 is also known as Nrg-1 β 3). Frequently in the literature one of the several recombinant EGF-like domains is used in isolation and referred to as Nrg-1 although the EGF-like domains are not expressed on their own in nature.

The first suggestion that Nrg-1 might have some role in the heart came from studies in mice genetically manipulated to remove functional Nrg-1 and its receptors. Mice with disrupted Nrg-1, ErbB 2, 3 and 4 receptors demonstrate the critical role of this signaling system in the development of ventricular trabeculation as well as the endocardial cushion required for formation of heart valves [5–7]. There are several interesting points that come from these studies. First is that while there are four Nrg genes, Nrg-2, 3 and 4 cannot supplant the requirement for Nrg-1 during cardiac development. It is also interesting that knockouts targeting specific exons of Nrg-1 demonstrate that a transmembrane Nrg-1 β is required for the heart to develop beyond this critical stage [7, 8]. The ErbB2 and ErbB4 knockout mice give the same phenotype as the Nrg-1 knockout [5, 9]. Thus collectively it appears that Nrg-1 β /ErbB2/ErbB4 represents a signaling cassette required for ventricular trabeculation during cardiac development. This work was quickly followed with studies of Nrg-1 and its receptors in the adult heart. Investigators found that this same cassette persists in the adult heart [10], where Nrg-1 β is expressed in the microvascular endothelial cell and activates both ErbB2 and ErbB4 on cardiac myocytes, leading to downstream activation of kinase cascades that can be demonstrated to modulate myocyte cell biology including cell growth, survival, as well as glucose uptake (see Figure 1, Table 2). Microvascular endothelial cells isolated from adult rat heart express the mRNA of at least 11 isoforms [11]. Both Nrg-1 α and Nrg-1 β are expressed, with the β isoforms being the most potent as activators of cardiac myocyte signaling and biological responses [11]. The majority of cardiac isoforms are expressed as transmembrane proteins that appear to require proteolytic activation, which has been observed in the setting of physiologic and pathologic stress [12, 13]. In some vascular beds Nrg-1 may have direct effects on endothelial cells inducing a proliferative response [14]. Thus cardiac Nrg/erbB signaling is a paracrine and juxtacrine system that regulates the interaction between microvascular endothelial cells and cardiac myocytes.

The early interest in understanding the role of Nrg-1 and its receptors in the adult heart was augmented by the observation that women with breast cancer treated with the erbB2-targeted antibody trastuzumab have increased risk of developing cardiac dysfunction and heart failure [15]. This finding provided important evidence for a role for erbB2 in the adult human heart, and motivated a number of laboratories to explore the role of neuregulin and its receptors in the heart. Subsequent studies demonstrated that ErbB2 and ErbB4 receptor signaling are required for maintenance of myocardial function in the adult heart, as cardiac specific deletion of functional receptors in mice leads to a dilated cardiomyopathy phenotype [16, 17]. Moreover, mice with conditional ErbB2 deletion or heterologous Nrg-1 deficiency have increased susceptibility to anthracycline cardiotoxicity [17, 18]. In adult myocytes in culture, anthracycline-induced sarcopenia is exacerbated by exposure to

antibodies against erbB2 and reduced by recombinant Nrg-1 β [19]. These observations demonstrate the critical role of Nrg-1 β /erbB signaling in the response of the heart to injury as well as the maintenance of normal myocardial structure and function.

Studies of Neuregulin/ErbB activity in human subjects

Using an enzyme-linked antibody assay, Nrg-1 β has been detected in human serum. In healthy subjects levels of Nrg-1 β correlate with fitness [20], which can be interpreted based upon animal work to be an indicator of physical activity, given that exercise is a potent activator of Nrg/erbB signaling in skeletal muscle [21]. In the setting of advanced heart failure, however, circulating Nrg-1 β is elevated in proportion to disease severity, particularly in those with ischemic cardiomyopathy [22]. While there are several interpretations of this observation, one possibility comes from the finding in animals that myocardial Nrg-1 β is released in response to ischemic injury [23] and neurohormonal activation [24]. This is supported by recent evidence for increased activity of Nrg-1 β /erbB signaling in the myocardium of animal models of heart failure [25]. Receptor down-regulation, a common feedback mechanism in biology, is also observed in chronic heart failure [26, 27], suggesting that heart failure could be a state of reduced Nrg-1 β responsiveness. Other evidence suggests that Nrg's play an important role in vascular health. Nrg suppresses macrophage migration into atherosclerotic plaques, and may be a negative regulator of atherosclerosis [28], perhaps accounting for the finding in the Cardiovascular Risk in Young Finns Study where a Nrg-1 polymorphism has been associated with atherosclerosis when coupled with stress [29].

Recombinant Neuregulin improves cardiac function in animals

Work with recombinant Nrg-1 β in the setting of animal models of chronic heart failure supports pursuing this as a therapy for systolic heart failure [30]. In rats with cardiac dysfunction induced by coronary artery ligation, a series of intravenous doses of a small fragment of Nrg-1 β (10 μ g/kg, IV, daily for 5 days) improved cardiac function (reduced left ventricular end systolic dimensions, improved fractional shortening, and ejection fraction). Similar improvements in cardiac function were seen in models of anthracycline and virally induced cardiac injury. In the anthracycline model Nrg-1 β was shown to act at least in part by protecting cardiac myocytes as evidenced by maintenance of cardiac troponin [31]. In dogs [30] and primates [32] with rapid-pacing induced heart failure Nrg-1 β induced improved cardiac contractility and relaxation.

As discussed, there are many effects of Nrg-1 β on the biology of cardiac myocytes and endothelial cells in the heart that could be contributing to the beneficial effects of Nrg-1 β on cardiac function (Table 2). In addition to protecting myocytes from stimuli that lead to loss of myocytes and progressive myocardial dysfunction, there are effects of Nrg-1 β on sarcomere gene expression, and sarcomere stability that implicate Nrg-1 β as a 'cardiac repair factor', improving cardiac function by allowing for repairing stressed cardiac myocytes. Alternatively, Nrg-1 β has also been reported to induce myocyte proliferation [33], implicating Nrg-1 β as a 'cardiac regeneration factor'. Whether Nrg-1 β -induced cardiomyocyte re-entry into the cell cycle is mechanistic in the improvement in cardiac function remains unknown. Still other potential mechanisms of the therapeutic Nrg-1 β action in animals are being defined, such as alterations in myocardial metabolism. Overall the data suggest that the effects of Nrg-1 β on the heart in disease models are pleiotropic including protection of contractile proteins, regulation of energy utilization, promotion of cell survival as well as cell division.

As will be discussed below, to our knowledge there are two forms of Nrg-1 β under development for the treatment of systolic heart failure. The 'EGF-domain only' fragment of

Nrg-1 β used by some investigators is not a natural product of the Nrg-1 gene, and lacks domains that are highly conserved in this family of proteins [4]. In particular, the Ig domain is expressed in Nrg-1 gene products, and is thought to mediate localization of ligand activity through interaction with matrix molecules. In *in vitro* assays of myocyte response to recombinant Nrg-1 β , we find that small EGF-domain only forms of Nrg-1 β have equal potency at activating intracellular signaling as the larger GGF2, but that the effects of GGF2 are longer-lasting [34]. In *in vivo* comparisons between these two peptides, GGF2 had more potent effects on improvement of cardiac function requiring less frequent dosing (Caggiano and colleagues, unpublished observation). Other studies have demonstrated that the Ig-like domain containing neuregulins are important for development and selective disruption of the Ig-like domain results in embryonic lethality with both cardiac and brain defects [35]. This may be explained in part by the differences in receptor binding and cellular signal transduction when comparing recombinant neuregulins with and without the Ig domains [36]. The potential relative benefits of the different Nrg-1 β isoforms as heart failure therapies have not been completely explored.

While recombinant Nrg-1 β improves cardiac systolic function after injury, there is no evidence that Nrg-1 β has a direct inotropic effect, increasing myocardial contractility through changes in calcium handling, excitation-contraction coupling, or myofilament calcium sensitivity. There have been no acute or delayed effects of recombinant Nrg-1 β on blood pressure or heart rate observed in numerous safety studies ([30]; our own unpublished data). In fact it appears that recombinant Nrg-1 β suppresses the increased fractional shortening induced by beta-adrenergic receptor activation [37, 38]. However there are effects of Nrg-1 β on calcium transients that suggest an effect on myocyte relaxation [39, 40]. Thus current data would suggest that Nrg-1 β acts via alterations in myocardial structure, and not via acute changes in calcium handling or EC coupling.

Early studies of recombinant Neuregulin in humans

How these multiple actions will translate into a potential human therapeutic is beginning to be explored. One Nrg-1 β fragment (a 61 amino acid EGF-domain only peptide common to all NRG-1 β) has advanced to clinical studies in Australia and China and another Nrg-1 β has begun early phase human trials in the United States. Preliminary results from a dose-ranging tolerability study with Nrg-1 β EGF domain peptide were recently reported [41]. Patients with systolic heart failure were treated for ten days with this Nrg-1 β , and after 90 days showed a trend towards improvement in cardiac structure and function as assessed by magnetic resonance imaging, at least at one dose studied. An analysis of acute hemodynamic effects of this peptide demonstrate that there is an acute increase in cardiac output that accompanies a vasodilator effect [42]. Of note, there was considerable nausea experienced by subjects receiving this peptide. Another issue that presents a challenge is the dosing schedule used in these trials, which has involved prolonged (multi-hour) infusion, daily, for multiple days. Larger Phase 2 and Phase 3 studies are ongoing in China and Australia [43, 44], and are registered but not yet activated in the United States [45]. The Phase 1 studies in patients with heart failure using a full-length glycosylated recombinant human Nrg-1 β 3 (also known as GGF2) have recently started [46]. Whether this longer acting Nrg-1 β will have any advantages in terms of safety, tolerability, and ultimately efficacy over the EGF-domain only peptide remains to be determined.

Questions and Problems for future research

As Nrg-1 β moves forward as a potential therapeutic for HF, it will be important to identify the mechanism for its action, and the extent to which systemic delivery of such a ligand with near ubiquitous receptor expression activates responses in other tissues. A concern in the development of Nrg-1 β as a therapeutic is the possibility that it will stimulate growth of

other tissue, including cancers [47]. Published data demonstrate the complexity of this issue with evidence of both the potential for tumor suppression as well as tumor growth acceleration[48, 49]. Another concern is that increased Nrg-1 β activity may have adverse effects on the central nervous system, as Nrg-1 β overexpression in the brain has been associated with behavioral changes in mice [50]. Thus it will be important to determine what dose/regimen is required to maintain any positive effects seen on cardiac function relative to the potential for risk of adverse effects. Ultimately the therapeutic benefit of Nrg-1 β must be established in well-controlled and sufficiently powered human trials reviewed and approved by Food and Drug Administration.

It is interesting to consider the many factors that have helped propel Nrg-1 β down the translational path. We consider three of these to be *interesting biology*, *diverse opportunity*, and *inverse evidence*. As highlighted in Table 2, *interesting biology* in the heart as well as other organs has attracted young scientists to this field, looking for worthwhile questions to investigate. There are still many important aspects of Nrg/erbB biology that remain to be answered; one can only hope that this is recognized by the many young scientists looking for areas to focus their efforts, and that funding agencies continue to support fundamental biological investigation. The *diverse opportunities* for developing Nrg's as therapeutics, as highlighted in Table 1, has provided an obvious stimulus for industry investment in moving Nrg's forward. Even if Nrg-1 β does not develop into a successful heart failure therapy, the lessons learned will certainly inform development of Nrg's for other disorders. Finally, progress in cancer therapeutics led to unexpected *inverse evidence* of the importance of erbB-signaling for maintenance of cardiac structure and function. This trastuzumab experience, and the collaborative investigations this stimulated focused on understanding the underlying mechanisms, have obviously helped to propel the development of erbB agonists as cardiovascular therapies forward. The rapid pace of oncology drug development is sure to create other such opportunities. Of course each of these factors has the attribute of stimulating interactions between people with different experiences and expertise, an excellent path to progress in any walk of life.

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Highlights

Neuregulin-1 β (Nrg-1 β) is a growth and survival factor in the heart and other organs.
Nrg-1 β works in cardiac myocytes via erbB2 and erbB4 receptor tyrosine kinases.
Nrg-1 β /erbB signaling in the heart regulates myocardial adaptation to stress.
Recombinant Nrg-1 β improves heart function in animal models of heart failure (HF).
Recombinant human Nrg-1 β is now being tested in humans as a possible HF therapy.

Figure 1.

Simplified schematic for actions of neuregulin and the erbB2 and erbB4 receptors in the heart where signaling are understood. Neuregulins are expressed in the adult heart in the microvascular endothelial cells, and act on adjacent myocytes by proteolytic release by a matrix metalloproteinase. Activation of erbB2 and erbB4 receptor tyrosine kinases leads to downstream activation of signals that regulate myocyte biology. While many of the details remain to be further elucidated, it appears that recombinant Nrg-1 β induces improved cardiac function via one or more of these biological responses.

Table 1

Adult tissues where NRG has been implicated including possible therapeutics

Tissue	Biological role	Potential therapeutic	Reference
CNS	Myelination	Multiple sclerosis	[51]
	Neuron protection & recovery	Stroke	[52, 53]
	Development	Schizophrenia	[54, 55]
Heart	Development and maintenance	Heart Failure	see text for references
Peripheral nerve	Myelination	Nerve injury	[56] [57]
Skeletal muscle	Synapse formation	Nerve and muscle injury	[58]
	Myoblast proliferation	Muscular dystrophy	[59]
	Glucose uptake	Metabolic syndromes	[60]
	Proliferation & Differentiation	Muscle injury	[61, 62]
		Wasting Syndromes	

Table 2

Biological effects of NRG in the heart

Cell type	Response	Signaling Pathway	Reference
Cardiac Myocyte	Survival, mitochondrial function	PI3-kinase/Akt	[10, 23, 63, 64]
	Glucose uptake, SR Calcium uptake	PI3-kinase/Akt	[11, 40]
	Growth	MEK/Erk	[65]
	Focal adhesion formation	Src/FAK	[66]
	Muscarinic Activation	?	[38]
	Cell Division	?	[33]
Stem cell	Myocyte differentiation	?	[67]
Endothelial Cell	Proliferation	?	[14]
Conduction System	Formation	?	[68]
Endothelial Progenitor Cells	survival	PI3kinase/Akt	[69]