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Review Article

Neuregulin-1β for the treatment of systolic heart failure

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ABSTRACT

The Neuregulin-1 gene encodes a family of ligands that act through the ErbB family of receptor tyrosine kinases to regulate morphogenesis of many tissues. Work in isolated cardiac cells as well as genetically altered mice demonstrates that neuregulin-1/ErbB signaling is a paracrine signaling system that functions in endocardial-endothelial/cardiomyocyte interactions to regulate tissue organization during development as well as maintain cardiac function throughout life. Treatment of animals with cardiac dysfunction with recombinant neuregulin-1beta improves cardiac function. This has led to ongoing early phase clinical studies examining neuregulin-1beta as a potential novel therapeutic for heart failure. In this review we synthesize the literature behind this rapidly evolving area of translational research. This article is part of a special issue entitled "Key Signaling Molecules in Hypertrophy and Heart Failure."

Contents

1. 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-1β (Nrg-1β) is one of these paracrine

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factors with interesting biological properties, and potential therapeutic value.

2. 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 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-1β3). 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-1β 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-1β/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-1β 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 Fig. 1, Table 2). Microvascular endothelial cells isolated from adult rat heart express the mRNA of at least 11 isoforms [11]. Both Nrg-1α and Nrg-1β areexpressed, 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, anthracyline-induced sarcopenia is exacerbated by exposure to antibodies against erbB2 and reduced by recombinant Nrg-1β [19]. These observations demonstrate the critical role of Nrg-1β/erbB signaling in the response of the heart to injury as well as the maintenance of normal myocardial structure and function.

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 Wasting Syndromes	[61,62]

3. Studies of Neuregulin/ErbB activity in human subjects

Using an enzyme-linked antibody assay, Nrg-1β has been detected in human serum. In healthy subjects levels of Nrg-1β 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-1β 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-1β is released in response to ischemic injury [23] and neurohormonal activation [24]. This is supported by recent evidence for increased activity of Nrg-1β/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-1β responsiveness. Other evidence suggests that Nrg's play an important role in vascular health. Nrg suppresses macrophage migration into atherosclerotic placques, and may be a negative regulator of atherosclerosis [28], perhaps accounding 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].

4. Recombinant Neuregulin improves cardiac function in animals

Work with recombinant Nrg-1β 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-1β (10 μg/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-1β was shown