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SPOTLIGHT REVIEW

Signalling between microvascular endothelium and cardiomyocytes through neuregulin

Emily M. Parodi and Bernhard Kuhn*

Harvard Medical School, Boston Children's Hospital, 300 Longwood Avenue, Enders Building, Room 1212, Brookline, MA 02115, USA

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Heterocellular communication in the heart is an important mechanism for matching circulatory demands with cardiac structure and function, and neuregulins (Nrgs) play an important role in transducing this signal between the hearts' vasculature and musculature. Here, we review the current knowledge regarding Nrgs, explaining their roles in transducing signals between the heart's microvasculature and cardiomyocytes. We highlight intriguing areas being investigated for developing new, Nrg-mediated strategies to heal the heart in acquired and congenital heart diseases, and note avenues for future research.

Keywords Neuregulin • Heart • Heterocellular communication • ErbB

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1. Neuregulins: structures and functions

Neuregulins (Nrgs) are a family of growth factors whose genes are principally identified by the presence of an exon coding for the epidermal growth factor-like (EGF-like) domain, which mediates the interaction of Nrg proteins with the v-erb-b avian erythroblastic leukemia viral oncogene homolog (ErbB) family of receptor tyrosine kinases.[1–4] Four Nrg genes are found in mammals, with partial family homology found in more distant relatives; Nrg orthologs are present in *Danio rerio*, *Xenopus laevis*, and *Drosophila melanogaster* genomes.[5–7] Nrg was first purified from neural tissue, where it was found to promote Schwann cell proliferation, and was thus named glial growth factor (GGF).[8,9] GGF was found to stimulate phosphorylation of the ErbB2 receptor tyrosine kinase, an effect linked to its mitogenic activity on Schwann cells.[10] Subsequent studies identified similar phosphoErbB2-stimulating proteins, which were eventually found to be isoforms encoded by a single gene, termed NRG-1.[11] The identification of three additional genes encoding similarly functioning isoforms has resulted in a variety of nomenclatures for members of the Nrg family. Nrg nomenclature includes the gene, the N-terminal sequence, the C-terminal sequence of the EGF-like domain, and finally, the cytoplasmic tail sequence. For simplicity, we use Nrgs to denote all isoforms of any of the four identified Nrg genes and specify the relevant Nrg gene and specific isoform when discussing protein products. NRG-1 is the most extensively studied gene, located on chromosome 8 in both humans and mice. NRG-1 encodes 21 exons[12,13] (*Figure 1A*) and has been suggested to give rise to as many as 31 potential protein isoforms.[14] N-terminal sequences distinguish Nrg1 isoforms as either Type I, Type II, Type III, Type IV, Type V, or Type VI[13,15] (*Figure 1B*). Nrg-1 amino terminal regions can include a signal peptide (sp), a kringle-like domain, a cysteine-rich domain, an immunoglobulin-like (Ig) domain, and a glycosylation region (*Figure 1B*). NRG-2, -3, and -4 exhibit far less diversity in isoform N-terminal sequences; NRG-2 encodes two N-terminal sequence variants, Type 1A and Type 1B (*Figure 1A*). Consistent among all Nrgs is the EGF-like domain, which mediates receptor binding, and can be classified based on the EGF-like domain's C-terminal sequence, which varies between α and β isoforms, each of which can exist in distinct variants. C-terminal to the EGF-like domain is a juxtamembrane (JM; also called stalk) region, which serves as the proteolytic cleavage site. C-terminal to the JM region is a transmembrane (TM) domain, followed by an a-, b-, or c-type cytoplasmic tail. The structures and functions associated with each of Nrg's domains provide important clues for understanding Nrg signal specificity. Kringle domains consist of triple-looped, 3-disulfide bridges,[16] are frequently found in clotting factors, and are proposed to serve as protein–protein interaction sites.[17] Ig-like domains are ~80 amino acid residues forming 7–10 β sheets and serve diverse cellular functions, including molecular transport, adhesion, morphogenic control, and cellular recognition.[18] The unifying region of all Nrgs, the EGF-like domain, is a protein domain comprised of six cysteine residues, which form 3-disulfide bonds. The EGF-like domain varies at its C-terminus as either an α or β variant, based on different exon usages.[19,20] *In vitro* studies have shown that Nrg-β isoforms are substantially more potent than Nrg-α isoforms;[21–24] however, this should not suggest that Nrg-α isoforms are biologically irrelevant, as Nrg-α isoforms have been demonstrated to be critically important for breast development[25] (*Figure 2B*).

* Corresponding author. Tel: +1 617 919 4712; fax: +1 617 731 0787. Email: bkuhn@enders.tch.harvard.edu

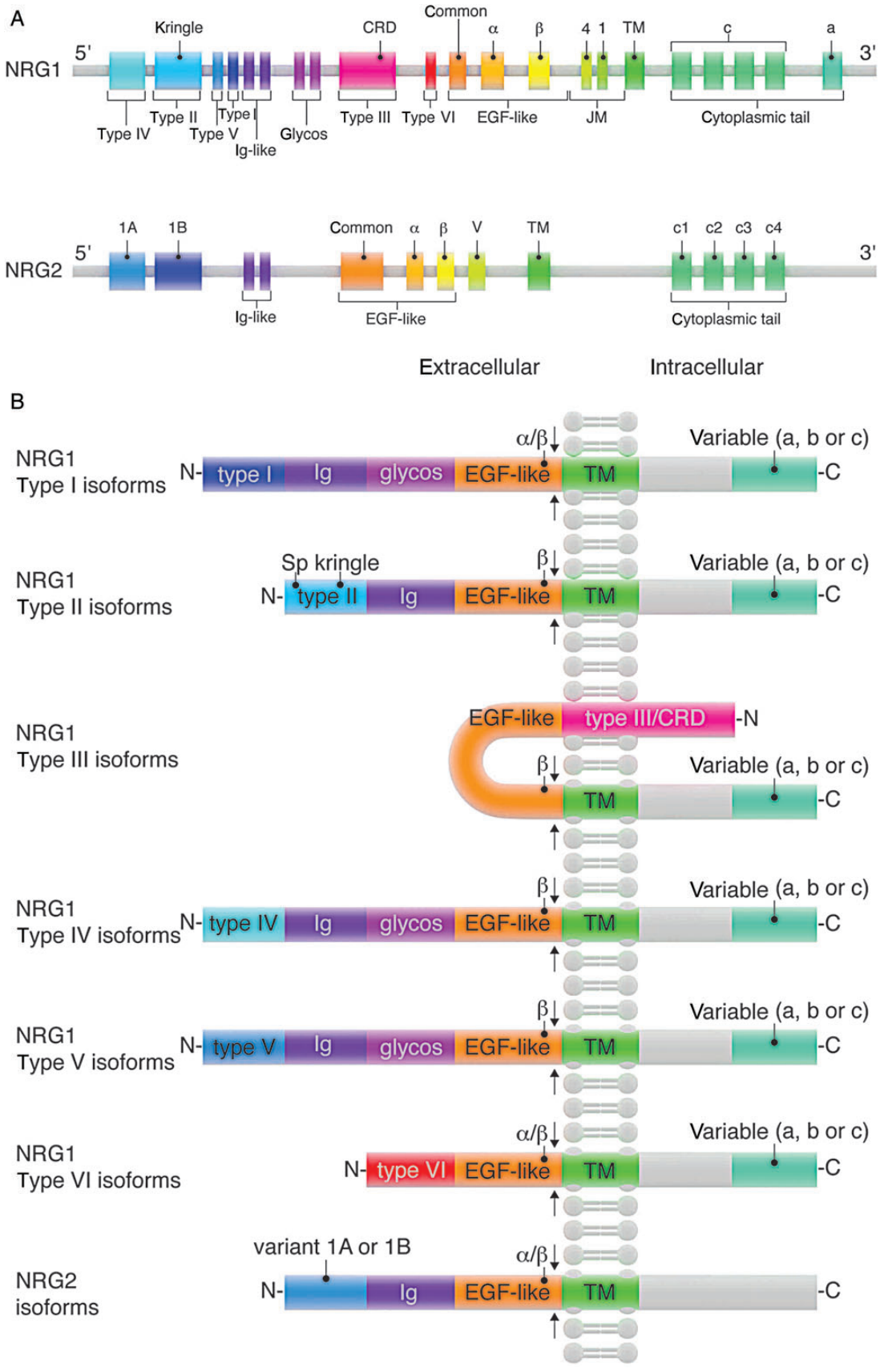

Figure 1 Nrg isoform structures: Nrg structures. (*A*) NRG-1 and NRG-2 genetic loci. (*B*) NRG-1 and NRG-2 protein isoform compositions. Arrows indicate putative proteolytic sites.

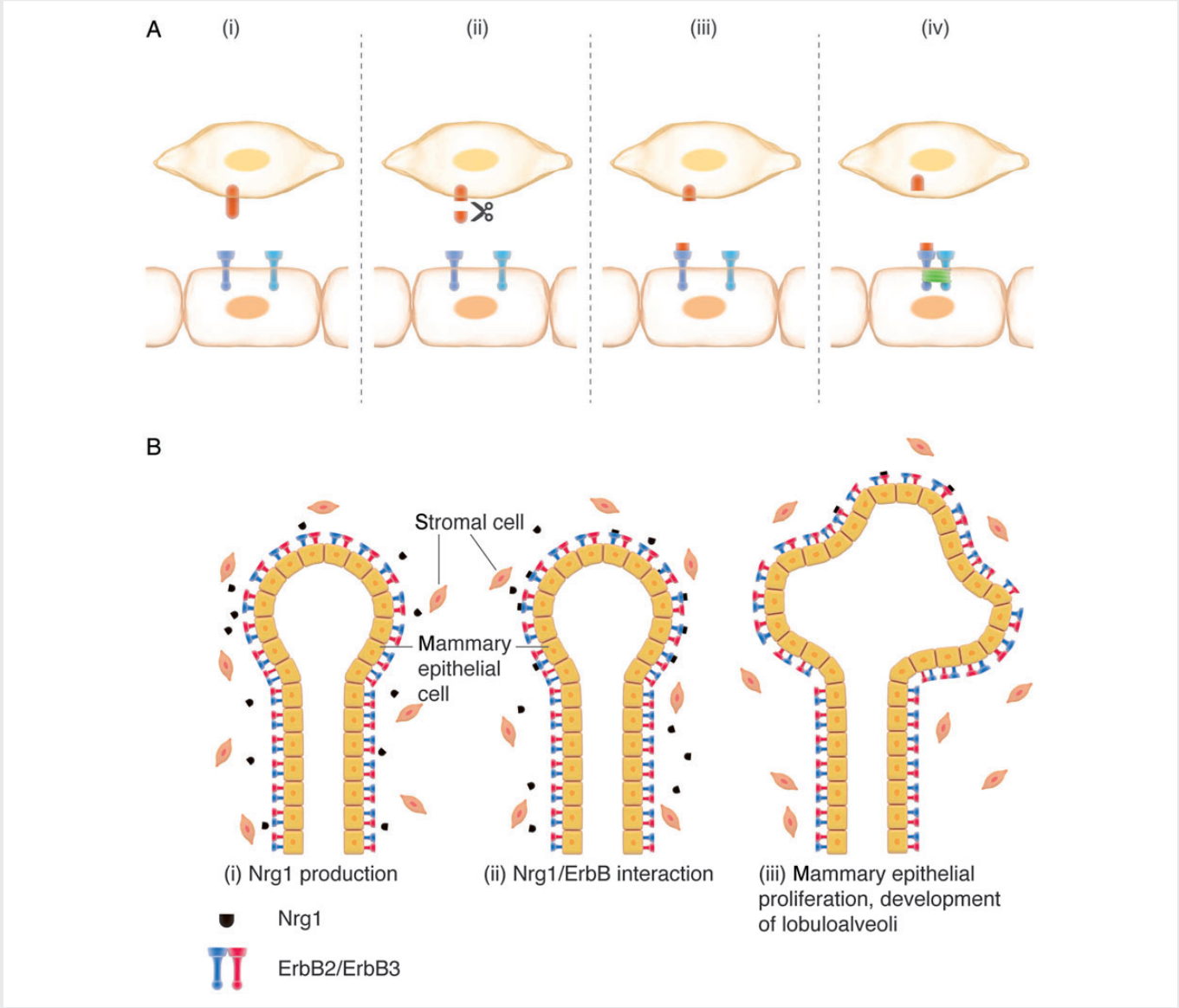

Figure 2 Nrg-1 heterocellular signalling: (*A*) Nrg heterocellular signalling between a coronary microvascular endothelial cell (top) and a cardiomyocyte (bottom). Scissor indicates proteolytic cleavage by metalloproteinases. The green box in (iv) indicates receptor phosphorylation and activation. (*B*) Nrg heterocellular signalling drives post-natal lobuloalveolar mammary gland changes upon pregnancy.

2. Nrg signalling specificity

Nrgs are expressed by a variety of cell types (*Table 1*), where they are either presented by the host cell and subject to cleavage for paracrine/juxtacrine signalling, or in the case of Nrg β-3 isoforms are instead directly secreted into the extracellular space.[47] Following release into the extracellular space, Nrgs bind ErbB receptor tyrosine kinases expressed on target cells. The ErbB receptor tyrosine kinase family consists of four members: ErbB1 (also known as epidermal growth factor receptor), ErbB2, ErbB3, and ErbB4. Nrg–ErbB binding causes receptor dimerization and phosphorylation (canonical Nrg/ErbB forward signalling), or receptor cleavage and internalization (non-canonical Nrg/ErbB forward signalling).[14,48–51]

Nrg domains control the release of Nrg into the extracellular space. Nrgs can be released by being directly secreted (β-3 Nrgs) or being presented on the cell surface for ectodomain release via proteolytic cleavage.[52] The JM region found between Nrg's EGF-like and TM domains serves as a proteolytic site for a disintegrin and metalloproteases (ADAMs).[53,54] ADAM17, also called tumour necrosis factor-α-converting enzyme (TACE), was shown cleave Nrg *in vitro*.[55,56] Nrgs can also be cleaved at the cytoplasmic tail by Bace1, a β-secretase, which releases Nrg's intracellular domain, which can translocate to the nucleus to promote expression of anti-apoptotic genes (reverse Nrg signalling).[57,58] Interestingly, Bace1 levels in the brain are elevated in congestive heart failure,[59] but it is unknown whether Bace1 levels similarly increase in the heart, which could implicate a systemic dysregulation of Nrg signalling in disease progression. Liu *et al.*[60] showed that truncation of the C-terminal tail in Nrg1 isoforms resulted in embryonic lethality with cardiac malformations, mimicking the phenotype seen in pan-Nrg1 knockouts. C-terminal truncation of Nrg1 cytoplasmic tails blocked proteolysis of the JM region, suggesting that the Nrg cytoplasmic tail plays a crucial role in mediating ectodomain release. Further support for the concept of Nrg proteolytic control via the intracellular domain is seen by studies, showing that Nrg

Table 1 Nrg-1 and Nrg-2 isoform expression

Gene	Isoform	Alias(es)	Receptor(s)	Prenatal tissue expression	Post-natal tissue expression	Disease overexpression	Cell types expressing
NRG-1	Nrg1 (Type unspecified)		ErbB4[26] ErbB3[27]	Lung[28,29]	Cornea[30], skin[31], skeletal muscle[32,33], motor trigeminal nucleus[2], breast[34]	Gut[35]	Pulmonary epithelial cells[36], fibroblasts[31], Golgi-II cells[2], cholinergic cells of basal forebrain[2]
	Nrg1 Type I	Acetylcholine receptor-inducing activity (ARIA)[37]; neu-differentiation factor (NDF)[38]; heregulin (HRG)[39]		Hindbrain[15], eye[15], dorsal spinal cord[15], cartilage in branchial arches[15], endocardium[15], trigeminal ganglion[15], telencephalon[15], superior/inferior colliculus[15], adrenal cortex[11], cortical neuroepithelium lining the lateral ventricles of the brain[11], brain above the developing thalamus[11], intestines[11], stomach[11], dorsal ganglia[11], genital ridge[11], liver[11], dermis[11]	Coronary microvasculature[23]		Neurons[40], astrocytes[40], cardiac microvascular endothelial cells[23,41]
	Nrg1 Type II	Glial growth factor II (GGF2)[10]		Spinal cord[15], dorsal root ganglia[15], skeletal muscle[15], entire brain[15]			Neurons[40] Astrocytes[40]
	Nrg1 Type III	Sensory and motor neuron-derived factor (SMDF)[42]		Cranial ganglia,[15] dorsal root ganglia,[15] ventral column of spinal cord,[15] entire brain,[15] olfactory epithelium[15]			Neurons[43], astrocytes[43], motor neurons[15]
	Nrg1 Type IV			Brain[44]	Hippocampus[44], prefrontal cortex[44]		
	Nrg1 Type V						
	Nrg1 Type VI						
NRG-2	Nrg2	Divergent of neuregulin-1 (DON1)[45]; neural- and thymus-derived activator for ErbB kinases (NTAK)[46]	ErbB4[2] ErbB3[2]	Telencephalon[46], brain[45], lung[45], endocardium[2]	Brain[2,46], olfactory bulb[2,46], cerebellum[2,46], thymus[46], brain[45], lung[45], cerebellum[45], denate gyrus of hippocampus[45], cerebellum[45], liver[2], spinal cord[2], hippocampus[2]		Neurons[46], granule cells[45], Purkinje cells[2,45], granule cells[2]

Tissue and cellular expression of Nrg1 and Nrg2 isoforms. Corresponding references are indicated in superscript.

ectodomain release was dependent on protein kinase C phosphorylation of a cytosolic Nrg residue.[61]

The Nrg/ErbB interaction that initiates heterocellular signalling cascades is a fascinating example of the diverse signalling achievable by a small number of genes. Neither ErbB1 nor ErbB2 bind any of the Nrg1, 2, 3, or 4 isoforms.[62,63] While ErbB3 can bind both Nrg1 and Nrg2 proteins, it is incapable of propagating the signal without heterodimerzation.[64,65] Only ErbB4 can autonomously respond to Nrg binding,[14] though like ErbB3 it also heterodimerizes. It has been suggested that dimer composition acts as a mechanism to regulate downstream signalling.[66] This theory is supported by studies showing that ErbB2 loss results in the absence of myocardial trabeculations, suggesting that despite the ability of ErbB4/ErbB4 homodimers to form, they do not sufficiently activate ErbB2/ErbB4-induced signalling cascades.[67–69] Additional signal diversification may be achieved at the ligand level, as *in vitro* studies have demonstrated that different Nrg's isoforms activate distinct downstream signal cascades; whether this mechanism is utilized *in vivo* remains to be determined.[70,71]

Although there are at least four identified genes encoding Nrg isoforms, only NRG-1 and NRG-2 isoforms have been shown to be expressed in the heart. Both NRG-1 and NRG-2 transcripts are detected in the embryonic endocardium, but only NRG-1 Type I isoforms continue to be expressed in the adult heart, being expressed in the endocardium and coronary microvasculature.[2,15,23,41,72] Receptors for the cardiac-specific Nrgs include ErbB2, ErbB3, and ErbB4.[63] Both ErbB2 and ErbB4 are expressed in the pre- and post-natal heart (*Figure 3*), where binding of Nrg1 to ErbB4 induces phosphorylation of itself and its co-receptor ErbB2.[41] ErbB3 is expressed in the developing atrioventricular cushions, while ErbB2 and ErbB4 are found in both developing and adult ventricles[41,73] (*Figure 3*).

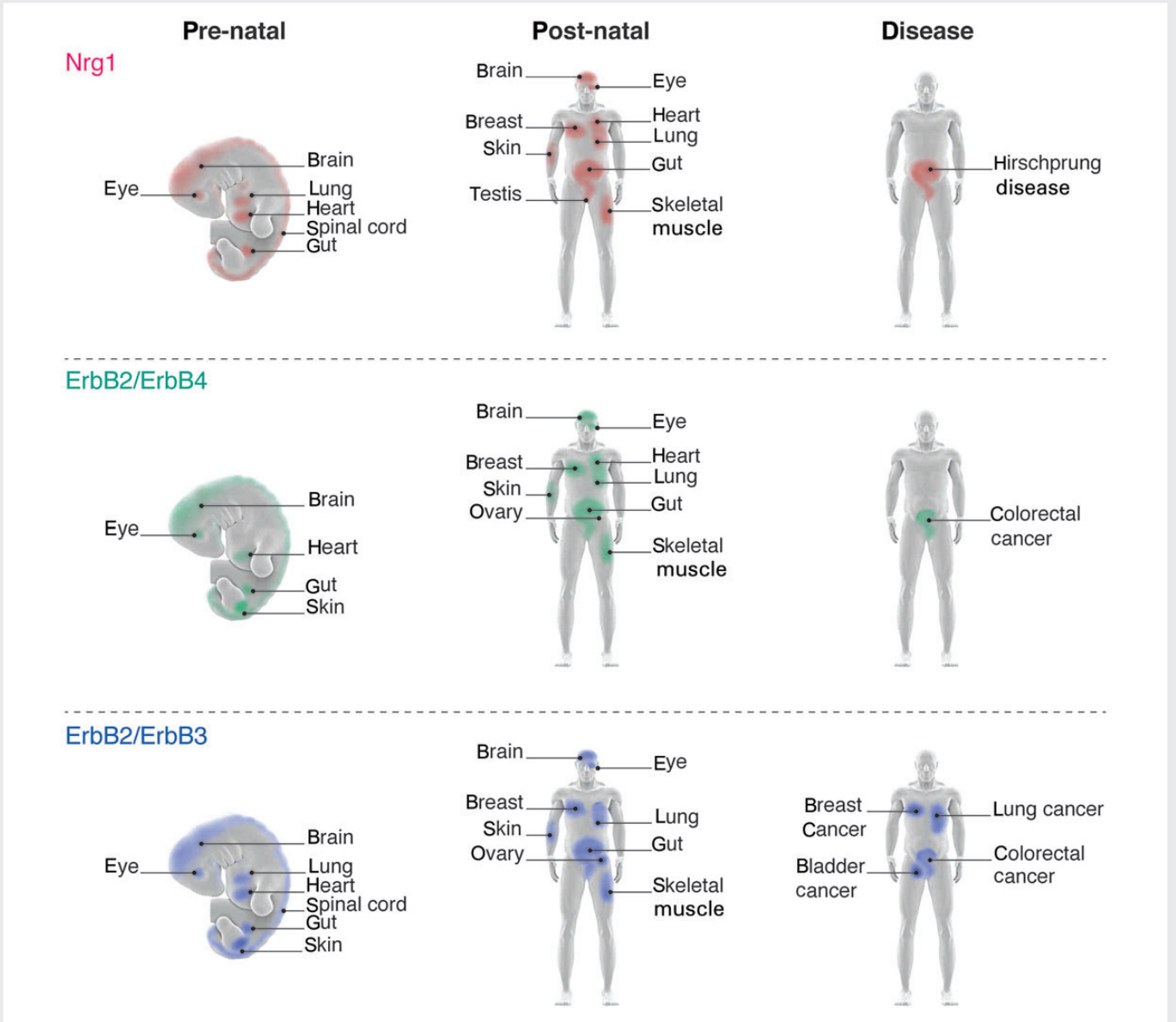

Figure 3 Nrg-1 ligand and receptor localizations: (top panel) tissues expressing Nrg1 during pre and post-natal life. (Middle panel) ErbB2 and ErbB4 expression profiles. (Bottom panel) ErbB3 and ErbB4 expression profiles. Disease expression indicates tissues overexpressing Nrg (top) or its receptors (middle, bottom) concomitant with indicated diseases.

Given the high level of signal modularity of the Nrg/ErbB interaction, it is not surprising that a number of downstream targets can be activated through this interaction. Among them are the Erk1/2, PI3K/Akt, and JAK/STAT signalling cascades.[74,75]

3. Nrgs in cardiac development, homeostasis, and disease

In the prenatal heart, Nrg1 and Nrg2 proteins are synthesized by cardiac microvascular endothelial cells.[23,41] Upon release into the extracellular space, Nrgs bind to ErbB4 receptors expressed in the myocardium. Binding of Nrg1 or Nrg2 to ErbB4 drives dimerization with ErbB2, resulting in a signal transduction cascade that promotes proliferation and differentiation of cardiomyocytes in the developing heart.[76] There is a clear delineation of specificity required to drive this proliferation, both on the level of ligand and receptor. Prenatal cardiac development requires Ig-containing Nrg1 proteins, evidenced by Ig-Nrg1 knockout mice dying at embryonic day 10.5 with aberrantly thin myocardial walls, indicating that non-Ig Nrgs (i.e. Type III and Type VI) are insufficient for cardiac development.[77] Interestingly, despite expression of Nrg2 proteins in the prenatal heart, they too appear to be insufficient to drive necessary levels of cardiomyocyte proliferation in the absence of Ig-Nrg1 proteins, a supposition further supported by the finding that loss of NRG-2 is not embryonically lethal, nor does it confer cardiac defects.[78] At the receptor level, loss of either ErbB4 or ErbB2 results in a phenotype highly similar to $Nrg1^{-/-}$, with both $ErbB4^{-/-}$ and $ErbB2^{-/-}$ mice failing to sufficiently grow and develop their myocardial walls.[67,68] Specifically, the reduction in myocardial thickness seen in loss of either $Nrg1^{-/-}$, $ErbB4^{-/-}$, or $ErbB2^{-/-}$ largely stems from the failure of these developing hearts to form ventricular trabeculations.[67,68,73] Levels of Nrg1 signalling in the heart must be tightly regulated, as ectopic expression of Nrg1 in the developing heart can drive hyper-trabeculation, a malformation known as ventricular non-compaction, which is associated with multiple congenital heart diseases.[76,79] Surprisingly, mice knocked out for TACE1 developed enlarged, hypertrabeculated hearts.[80] Why loss of TACE1 results in a phenotype dissimilar to that seen in Nrg1, ErbB2, and ErbB4 knockouts is not fully understood, though it is speculated that TACE1 loss results in hyperactivation of ErbB4, resulting in increased cardiac proliferation and trabeculation.[80]

ErbB3 is expressed in the developing, but not in the adult, heart and is detected in the invading mesenchyme and endocardial cardiac cushions.[41,81] This distinct localization is reflected in the aberrant cardiac cushions formed in $ErbB3^{-/-}$ mice.[81,82] In addition to driving proliferation and differentiation of working cardiomyocytes, Nrg1 proteins also promote the appropriate differentiation and recruitment of contractile cardiomyocytes to the cardiac conduction system.[83]

In the adult heart, Nrgs are expressed in response to physiological stress; endogenous and exogenous administration of NRG-1 has unveiled several cardioprotective and cardioregenerative functions of NRG-1, which include protection against: apoptosis, myofibrillar disarray, anthracycline-induced cardiomyopathy, and scar formation[84–87] as well as proliferation of post-natal cardiomyocytes. These effects are summarized in *Table 2*. Interestingly, NRG-1-induced protection from myofibrillar disarray has been shown *in vitro* and *in vivo*;[92,100] however, these findings contrast with the lack of myofibrillar disarray observed in ErbB2 conditional knockouts.[102,103] The dichotomy seen between these gain-of-function and loss-of-function studies highlights an intriguing area for future research and suggests a potential role for ErbB4 homodimers in NRG-1-induced cardioprotection.[86]

NRG-1 has also been suggested to play a role in cardiac hypertrophy; however, like myofibrillar disarray, NRG-1's relationship with this cardiac effect is not fully understood. Administration of NRG-1 to cardiomyocytes *in vitro* has been shown to increase hypertrophy[74] in neonatal rat ventricular cardiomyocytes; however, administration of NRG-1 following left anterior coronary artery (LAD) ligation was shown to decrease cardiomyocyte hypertrophy.[87] Further complexity is seen in the increase of NRG-1, ErbB2, and ErbB4 expression coincident with cardiac hypertrophy, all of which rapidly decline in expression upon transition to heart failure.[104] These collective findings suggest a complex relationship between NRG-1 and cardiac hypertrophy, likely involving both feed-forward and feed-back signalling pathways, and highlight another important avenue for future research.

The favourable effects which Nrg administration confers upon the heart are shared by other identified cardioprotective and cardioregenerative growth factors. Like NRG-1, administration of insulin-like growth factor 1 (IGF-1), periostin peptide, or fibroblast growth factor 1 (FGF-1) is sufficient to induce post-natal cardiomyocyte cycling.[87,105–107] While FGF-1 and IGF-1 therapies result in increased cardiac hypertrophy,[108] periostin peptide and NRG-1 therapies have an opposite effect, with administration of either growth factor resulting in decreased cardiomyocyte hypertrophy in adult mice or rats after experimental myocardial infarction (MI).[87,106,109] Additionally, like NRG-1, IGF-1, FGF-1, FGF-2, urocortin, vascular endothelial growth factor (VEGF), transforming growth factor beta-1, and cardiotrophin therapies all are associated with decreased apoptosis in the heart.[86,90,110–122] It is noteworthy that NRG-1's apoptotic protection appears to be context-dependent in animal models. For example, while NRG-1 administration following LAD ligation has no effect on apoptosis,[87] both NRG-1 treatment for diabetic cardiomyopathy and bivalent NRG-1 administration following anthracycline-induced cardiotoxicity result in attenuation of cardiomyocyte apoptosis.[86,88,90,110,111] Similar to NRG-1, periostin peptide administration following LAD ligation has no effect on apoptosis; it is important to note, however, that unlike NRG-1, periostin peptide was administered immediately following LAD ligation, in contrast to NRG-1 being administered 1 week following LAD ligation.[106] The parallels seen between NRG-1 and other growth factors conferring cardiac protection and regeneration are likely therefore to elucidate additional therapeutic potentials for cardiac growth factors.

The importance of endogenous NRG-1 in post-natal cardiac homeostasis is further demonstrated by the resultant cardiotoxicity in patients receiving the breast cancer chemotherapeutic Herceptin, which targets the NRG-1 receptor ErbB2.[123] These findings are in line with studies showing that conditional ErbB2 deletion in mice results in dilated cardiomyopathy,[102,103] although the detailed cellular mechanisms of Herceptin-induced cardiomyopathy remain to be elucidated.

The importance of NRG-1 in the post-natal heart is further demonstrated by the studies showing alterations in Nrg signalling being correlated with some cardiac diseases. In a rat model of cardiac hypertrophy induced by aortic constriction, Nrg-1 transcript levels remained constant, while ErbB2 and ErbB4 transcript and protein levels significantly decreased.[104] Similarly, myocardium from heart failure patients displayed normal Nrg levels, but had extremely decreased ErbB2 and ErbB4 levels.[124] These findings suggest abnormalities of receptor regulation in the failing myocardium.

In contrast, heart failure induction via pacing in dogs showed a substantial increase in Nrg1, ErbB2, and ErbB4 RNA levels, with

Table 2 Cardiac effects of Nrg-1 isoforms

Neuregulin	Effects	*In vitro*	*In vivo*
Nrg1, rhNRG-1	Improvement of myocardial function in diabetic cardiomyopathy		+[88]
Nrg1, rhNRG-1	Protection against diabetic cardiomyopathy-induced cardiomyocyte apoptosis	+[88]	
Nrg1 Type I β, rhNRG, AA 1-241	Reduction in anthracycline-induced alterations of EC coupling	+[89]	
Nrg1 Type II β, rhGGF2	Cardiomyocyte proliferation	+[41]	
Nrg1 Type II β, rhGGF2	Cardiomyocyte survival	+[41]	
Nrg1 Type II β, rhGGF2	Cardiomyocyte hypertrophy	+[41]	
Nrg1 Type II β, GGF2	Protection of cardiac myocytes from anthracycline-induced apoptosis	+[90,91]	
Nrg1 α	Prevents anthracycline-induced myofilament injury	−[92]	
Nrg1 α2, EGF-like domain	Drives negative inotropic response	+[93]	
Nrg1 β	Prevents anthracycline-induced myofilament injury	+[92]	
Nrg1 β, rhGGF2	Protects against anthracycline-induced cardiotoxicity		+[91,94]
Nrg1 β, EGF-like domain	Angiogenesis	−[95]	+[95]
Nrg1 β (extracellular domain including the Ig-domain)	Angiogenesis	−[95]	
Nrg1 β1, AA 177-241	Angiogenesis	+[96]	
Nrg1 β3, EGF-like domain	Angiogenesis	+[96]	+[96]
Nrg1 β	Reduced hypertension	N/A	+[97]
Nrg1 β, GGF2	Improve systolic function	N/A	+[98]
Nrg1 β (bivalent)	Attenuation of anthracycline-induced decrease in fractional shortening	N/A	+[86]
Nrg1 β, EGF-like domain, AA176-246	Cardiomyocyte proliferation	+[87]	+[87]
Nrg1 β, EGF-like domain, AA176-246	Cardiomyocyte proliferation following MI	N/A	+[87]
Nrg1 β, EGF-like domain, AA176-246	Differentiated cardiomyocyte cell-cycle re-entry	+[87]	+[87]
Nrg1 β, EGF-like domain, AA176-246	Improvement of cardiac structure after MI	N/A	+[87]
Nrg1 β, EGF-like domain, AA176-246	Improvement of cardiac function after MI	N/A	+[87]
Nrg1 β1	Improved Ca^{2+} handling	+[99]	
Nrg1 β2a, AA Ser177-Glu237	Prevents anthracycline-induced myofilament injury	Data not shown[100]	
Nrg1 β2a, AA Ser177-Glu237	Protects against anthracycline-induced cardiotoxicity		+[100]
Nrg1, rhNRG-1	Prevention of Angiotensin-II-induced diastolic dysfunction	N/A	+[101]
Nrg1, rhNRG-1	Reduction in Angiotensin-II-induced cardiac hypertrophy	+[101]	+[101]
Nrg1, rhNRG-1	Reduction in Angiotensin-II-induced myocardial fibrosis	+[101]	+[101]

Plus indicates positive effect and minus indicates negative effect. Corresponding references are indicated insuperscript.
MI, myocardial infarction; EC, excitation–contraction.

accompanying induction of the Nrg1/ErbB-activated PI3K pathway, but not Erk or Akt activation, cardioprotective pathways normally activated by Nrg1/ErbB signalling.[125] Genetic screening in congenital heart disease patients revealed an association between an ERBB4 haplotype and defects of the left ventricular outflow tract.[126] The dichotomy of Nrg1 expression in different models of heart failure may be a result of disease specificity, with differing maladaptive mechanisms being activated in the different diseases. Such a possibility could explain the distinct Nrg1 and ErbB expression patterns seen in a rat model for diabetic cardiomyopathy, wherein both Nrg1 and ErbB2 as well as ErbB4 are decreased, a phenotype distinct from either disease expression pattern described above.[127] Similarly, Nrg is detectable in both plasma and sera from heart failure patients, although it is differentially associated with disease severity depending on the heart failure type,[128,129] further supporting the concept of differential Nrg regulation based on the pathogenic mechanism.

Endogenous Nrgs may also promote cardiac function by acting on non-cardiomyocytes. NRG-1 expression promotes cardiac function following injury via induction of angiogenesis. Endothelium-derived Nrg was shown to promote angiogenesis both *in vitro* and *in vivo*, and reduction of Nrg was found to correlate with a reduction in angiogenesis following ischaemic injury, further supporting the role of Nrg as a pro-angiogenic factor.[84,96,130] Intriguingly, Nrg-stimulated angiogenesis has been reported to be independent of VEGF;[96] however, a different study challenges such VEGF-independence, finding that Nrg can drive angiogenesis by stimulating expression of VEGF.[95]

NRG-1 cardioprotection via non-cardiomyocytes may also involve actions upon cardiac fibroblasts, as NRG-1 administration following injury results in a decrease in scar size.[87] Whether NRG-1 acts directly or indirectly on fibroblasts to drive this effect is unknown, and highlights another intriguing area for future mechanistic studies into how NRG-1 drives cardioprotection.

How Nrg expression is stimulated in the adult heart remains an intriguing area of investigation. Studies have found that enodthelin-1 and increased mechanical stress on the heart increase Nrg expression, whereas angiotensin-II and phenylephrine decrease Nrg expression;[131]

this suggests an important mechanism for regulation of Nrg signalling at the ligand level by sensing of alterations to cardiac demand. The finding that increased mechanical stress on the heart increases Nrg expression is intriguing, as integrins, a family of receptors expressed on many cells, including cardiomyocytes, act as sensors of mechanical stress as well as potentially as non-canonical receptors for Nrg1 proteins.[132,133] The concept of stress driving positive regulation of Nrg expression is further supported by studies, showing that Nrg expression in the heart is increased during pregnancy, a time when haemodynamic stress increases cardiac demand.[134]

4. Therapeutic administration of Nrg: potentials and challenges

Given the critical requirement for Nrgs in cardiovascular development, as well as their ability to drive cardioprotective downstream signalling cascades, it is not surprising that administration of recombinant Nrg isoforms have been identified as cardiac beneficial agents with potential applications in clinical management of heart diseases. To date, ClinicalTrials.gov has listed seven clinical trials examining NRG-1 administration as a cardiac therapy. Current clinical trials for NRG-1 therapy are limited for the treatment of systolic dysfunction; it is likely that future trials may investigate the feasibility of NRG-1 therapy for the treatment of diastolic dysfunction, as animal models of diabetic cardiomyopathy- and Angiotensin-II-induced injury have shown that NRG-1 administration can attenuate diastolic dysfunction.[101,110] Given the high incidence of diastolic dysfunction,[135–137] future mechanistic research into possible different means and disease contexts in which NRG-1 treatment can attenuate such dysfunction presents an important area of research. Furthermore, NRG-1 has only been investigated as therapeutic treatment in dilated and hypertrophic cardiomyopathies. Whether NRG-1 treatment can improve restrictive and arrhythmogenic cardiomyopathies is unknown, and presents another intriguing area for future studies.

Nrg's cardiac benefits *in vivo* include its ability to decrease scar size and improve cardiac function following MI.[87] A complete picture of the ways Nrg drives such cardiac benefits is still being elucidated; our laboratory has found that Nrg increases cardiomyocyte number, rather than size, following infarct; however, it is likely that this is not the sole mechanism responsible for the functional improvements by Nrg in acute heart failure. Gu *et al.*[138] demonstrated that increased cardiac myosin light chain kinase (cMLCK) expression and regulatory light chain phosphorylation accompanied post-Nrg improvement of function in hearts; however, Chang *et al.*[139] found that suppression of the cMLCK expression did not mitigate Nrg's cardiac benefits, suggesting alternative pathways utilized.

A critical question to answer for the advancement of Nrg therapy is how, when, and in what disease types, Nrg should be administered. Most studies have examined Nrg in a cardiac beneficial role with administration following injury. Pre-treatment with Nrg under *ex vivo* conditions was found to increase scar formation; this, however, is in contrast with an *in vitro* study showing Nrg promoting cardiomyocyte survival.[84,140]

Nrg has also been investigated as a therapeutic to be co-administered to cancer patients receiving anthracycline therapy. *In vivo* studies have shown that administration of Nrg prior to and during anthracycline treatment helps protect against anthracycline-induced cardiotoxicity,[86,91,94,100] suggesting promise for Nrg as a co-therapeutic for cancer patients receiving anthracycline therapy. Engineered bivalent Nrg has been shown to drive protection from anthracycline-induced cardiotoxicity,[86] and it presents another means with which the diverse Nrgs can be utilized to protect the injured heart.

The promise of Nrg in clinical management of heart failure is potentially mitigated by one of Nrg's own great strengths—its mitogenic potential. Although ErbB2 does not directly bind to Nrgs, ERBB2 gene amplification leading to overexpression is a key feature of many cancers, the best documented of which is breast cancer (reviewed in[141]). The finding that expression of one component of the Nrg signalling system is increased in some cancers has led to the supposition that therapeutic application of recombinant NRG-1 for cardiac therapy may unintentionally stimulate proliferation of non-cardiac cells. Close examination of ErbB2's function in promoting tumorgenicity reveals that ErbB2-assosciated tumorgenicity is often Nrg-independent. Studies have shown that overexpressed ErbB2 found in tumours is frequently a mutant version, which is constitutively active in the absence of a ligand.[142,143] Additionally, silencing of the NRG-1 locus is a hallmark of many breast cancers,[144] though a genetic NRG-1 translocation that produces secreted Nrg1 is associated with other breast cancers,[145–149] suggesting that the capacity for Nrg to induce and progress malignancy is tightly tied to biological context. Direct binding partners for Nrgs, ErbB3 and ErbB4, have also been associated with cancer via incidence of their up-regulation in cancers; however, assignment of causality may be premature, as overexpression of either receptor is associated with both improved[150] and reduced[151] cancer prognosis.

Another concern for clinical use of NRG-1 for cardioprotection is whether NRG-1 therapy will specifically activate its intended cardiac receptors. As NRG-1 is administered systemically, it is possible that its post-natal receptors throughout the body may be activated and promotes adverse reactions. Data from clinical trials revealed two incidences of skin cancer, as well as complaints of nausea;[152] while another trial reported no significant side effects.[153] It is important to note that the two incidences of skin cancer were concluded to have been pre-existing conditions. It is likely that the relationship between NRG-1 isoform specificity and receptor activation accounts for the minimal adverse side effects reported; however, publication of results from other clinical trials will be important in assessing the potential of NRG-1 systemic administration. If NRG-1 systemic administration is determined to be unfeasible in certain patient populations, alternative means of delivery would present important therpautic alternatives. Our laboratory has demonstrated that interpedicardial gel-foam delivery of periostin peptide allows for cardioprotection without systemic administration;[154] similar drug delivery of NRG-1 could be utilized in potential patient populations restricted for systemic NRG-1 adminstration.

Reduced Nrg levels are also associated with an increased risk for the development of schizophrenia;[155] however, as the link between Nrg and schizophrenia is based on reduction in basal Nrg levels, administration of exogenous Nrg for cardiac dysfunction is not expected to raise risk for schizophrenia. Despite this, given the fine modulation of signalling in both cardiac and neuronal systems, as well as the ability for some Nrgs to cross the blood–brain barrier,[43,156,157] clinical studies evaluating Nrg as a cardiac therapy should probably include cognitive and behavioural monitoring.

Inappropriate Nrg signalling is also linked to Hirschsprung disease, a congenital disorder characterized by a regional lack of innervation in the gut, which prevents gut motility. Increased Nrg, both at the transcript and protein levels, was found in the intestines of Hirschsprung disease patients,[35] suggesting that inappropriate Nrg signalling is a causal factor in this disease.

5. Conclusions

The genetic architecture of Nrgs and their receptors allows for diverse isoforms to be expressed. The diverse isoforms have both overlapping and distinct requirements, reflecting the complexity of the Nrg/ErbB signalling system. The diverse effects achievable by Nrgs are beginning to be harnessed for clinical therapies, with the Nrg's multiple cardiac effects showing great promise for treating heart failure. Continued elucidation of Nrg signalling shows great promise for not only clinical therapies, but also for better understanding of the pathogenesis and progression of heart failure.

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