Neuregulin-1, a microvascular endothelial-derived protein, protects against myocardial ischemia-reperfusion injury (Review)

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Abstract. As regards acute myocardial infarction, great success has been achieved in therapies that reduce the effects of myocardial ischemic injury, while few interventions have achieved satisfactory outcomes for myocardial ischemia-reperfusion (IR) injury. Thus, new research is urgently required to achieve breakthroughs in promising treatments. Neuregulin-1 (NRG-1), which is an endothelium-derived protein and the ligand of ErbB receptors, exerts cardioprotective effects and is rapidly upregulated during IR. NRG-1/ErbB activates several downstream signaling pathways in response to myocardial IR injury. Previous studies have revealed the protective effects of NRG-1 during heart failure, and numerous experiments have explored the mechanisms underlying the NRG-1-induced cardioprotective effects against myocardial IR injury. In the present review, the progress made in the research of NRG-1 as a cardioprotective agent during IR and related conditionings is summarized. Furthermore, the potential benefits of NRG-1 against myocardial IR injury are listed with the prospective use of NRG-1 in clinical applications.

Contents

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1. Introduction

Acute myocardial infarction (AMI) is the leading cause of morbidity and mortality worldwide (1). Timely reperfusion is crucial for salvaging the ischemic myocardium. However, the rapid restoration of blood flow into the myocardial tissue following a period of ischemia may lead to additional tissue damage, termed myocardial ischemia-reperfusion (IR) injury, resulting in an increase in the myocardial infarct size and a decrease in cardiac function (2). The mechanisms responsible for myocardial IR injury are intricate, involving an excessive inflammatory response, endoplasmic reticulum stress, calcium overload, oxidative stress and cardiomyocyte death (e.g., autophagy, apoptosis and necroptosis) (3).

Neuregulin-1 (NRG-1), a stress-mediated growth factor that binds cardiomyocyte tyrosine kinase receptors of the erythroblastic leukemia viral oncogene (e.g., ErbB2 and ErbB4), is mainly synthesized and secreted by endocardial and microvasculature endothelial cells in the heart (4). Upon ligand binding, activated NRG-1/ErbB signaling directs cell fates (e.g., survival, migration, differentiation and proliferation) (5). Currently, the therapeutic potential of NRG-1 in cardiovascular diseases is gradually being revealed. Numerous studies have demonstrated that NRG-1 protects myocardial cells from injury and restores cardiac functions, such as protection against doxorubicin (DOX)-induced cardiomyocyte toxicity (6) and anti-fibrotic and anti-remodeling effects in heart failure models (7). Notably, the administration of recombinant human NRG-1 has been shown to significantly improve cardiac function in phase I and II clinical trials of heart failure (HF) (8). Given the beneficial effects of NRG-1 on cellular survival and cardiac function, the role of NRG-1 in myocardial IR injury should be of interest to researchers. Furthermore, the protective mechanisms of NRG-1 share common features with myocardial IR injury. On the other hand, myocardial IR

injury is one of the major pathophysiological conditions in the pathogenesis of HF, and effective treatments with IR injury could hamper the onset and development of HF.

2. Overview of NRG-1

Structure and isoforms of NRG-1. As a member of the Neuregulin family of growth factors, NRG-1, encoded by a gene spanning 2.4 million base pairs in mice and 2.6 million base pairs in humans and rats, is located on chromosome 8 in humans and mice, and on chromosome 16 in rats (9,10). The 6 known types of NRG-1 proteins (types I-VI) are classified by 6 different transcriptional initiation sites, and alternative splicing of the NRG-1 gene produces 33 different isoforms in humans (11,12). The 6 types of NRG-1 are defined by differences at the N-terminus; more specifically, these differences lay in the linker region between the transmembrane domain and the EGF-like domain encoded by exons (13,14). All 6 isoforms have an EGF-like domain, which is necessary and sufficient for the activation of ErbB receptors. However, only type I, II, IV and V isoforms have an additional immunoglobulin (Ig)-like domain, which is located between the N-terminal sequence and the EGF-like domain (Fig. 1A), while NRG-1 types III and VI are characterized by an N-terminal region connected directly to the EGF-like domain (Fig. 1B) (10,14). Of note, the N-terminal region of NRG-1 type III consists of a cysteine-rich domain (CRD) and an additional N-terminal transmembrane domain (TM), and this unique structure limits the functional fragment release (Fig. 1C) (14). NRG-1 protein is functionally shed by proteolytic cleavage of its membrane-bound precursor (pro-NRG-1) (15). A bioactive extracellular NRG1 fragment is released by type I transmembrane domain proteases [e.g., beta-secretase (BACE), a disintegrin and metallopeptidase domain (ADAM)17, ADAM19 and ADAM10], termed mature NRG-1, acting in a juxtacrine or paracrine manner, while mature NRG-1 type III is anchored by CRD rather than shedding from the membrane (16).

ErbB receptor and associated signaling pathways. To exert vital biological functions, NRG-1 must bind to a family of ErbB to activate tyrosine kinase receptor proteins. The ErbB family encompasses four transmembrane tyrosine kinase receptors: ErbB1 [also termed epidermal growth factor receptor (EGFR)], ErbB2, ErbB3 and ErbB4 (10,13). Upon ligand binding, an ErbB receptor encounters structural modifications in the juxta-membrane domain (JMD) (17), which leads to the homodimerization or heterodimerization of ErbB receptors due to differences in receptor affinity. NRG-1 ligand binding triggers the homodimerization of ErbB4 and the heterodimerization of ErbB-2/3, ErbB-2/4 and ErbB-3/4 (18). Compared with NRG-1, EGF has more potent affinity to the binding site of ErbB1 (19). Among the ErbB receptors, only ErbB4 can form functional homodimers upon NRG-1 binding, as it consists of both ligand binding and active kinase domains (15). On the other hand, ErbB2 lacks the ligand binding pocket, while ErbB3 only has a pseudokinase domain. The absence of sufficient signal transmission requires the heterodimerization of both receptors to exert their function (13).

The dimerization of ErbB receptors (either homo- or heterodimerization) activates the tyrosine kinase domain and induces the phosphorylation of the cytoplasmic region of the ErbB partner (20). Subsequently, the phosphorylated tyrosine residues recruit various adaptors/effectors and trigger a switch in signal transmission. The main activated downstream signaling molecules are the phosphoinositide 3-kinase (PI3K)/Akt and Raf/MEK/extracellular regulated kinase (ERK) pathways (21), which are both important reperfusion injury salvage kinases (RISK). Other downstream kinases known to be activated by NRG-1 include Pyk2, c-Abl, JNK, Fyn and CDK5 (10). In the cardiovascular system, these pathways affect cell survival, migration, adhesion and differentiation properties and proliferation (22), and they play an indispensable role in maintaining cardiovascular homeostasis when cardiomyocytes encounter stimuli or insults, such as hypoxia, acidosis and oxidative stress, that contribute to myocardial IR injury.

3. NRG-1: Related mechanisms involved in myocardial IR injury

The NRG-1/ErbB pathway is considered a compensatory protective mechanism of cardiac insult (23). It has been reported that the cardiac NRG-1/ErbB pathway is upregulated following myocardial IR. Fang *et al* observed the significant upregulation of NRG-1 at both the mRNA and protein levels, which was accompanied by a marked increase in the phosphorylation of the ErbB4 receptor in the IR-affected myocardium (24). Morano *et al* also found that the expression of ErbB3 was upregulated upon ischemic injury (25). It is generally recognized that excessive reactive oxygen species (ROS) are important mediators of reperfusion injury. Accumulated ROS has been shown to upregulate the NRG-1 secretion and the phosphorylation of ErbB (26), which can also be induced by hypoxia (27).

Thus, myocardial ischemia appears to be involved in the upregulation of NRG-1/ErbB in the heart following IR, and the core part of this upregulation may be the accelerated generation of ROS, which is attributed to adenosine triphosphate (ATP) deficiency, hypoxia, mitochondrial permeability transition pore (mPTP) opening and mitochondrial damage. Since the attributions of IR injury are not individual segments but complex networks, and the association between NRG-1 and IR injury is not a single unidirectional link, in the present review, the effects of NRG-1 via the pathways from related triggers of ROS to eventual cell death during IR are illustrated (Fig. 2).

Excessive inflammatory response. With the initial activation of inflammation accompanied by the release of pro-inflammatory cytokines, as well as inflammatory leukocyte recruitment and infiltration, the heart attempts to defend itself against deleterious stimuli during IR. However, excessive inflammation in the endangered myocardial region can lead to exacerbated myocyte death via pro-apoptotic signaling pathways and further cardiac remodeling (28,29). Toll-like receptors (TLRs) and nuclear factor-κB (NF-κB) are the primary pathways associated with IR-induced inflammation (3). TLRs are expressed by inflammatory cells, endothelial cells and cardiomyocytes. In addition to defending against microorganisms, TLRs, particularly TLR-4, play a critical role in inflammation-induced apoptosis during IR (30). NF-κB pathways can be divided into canonical

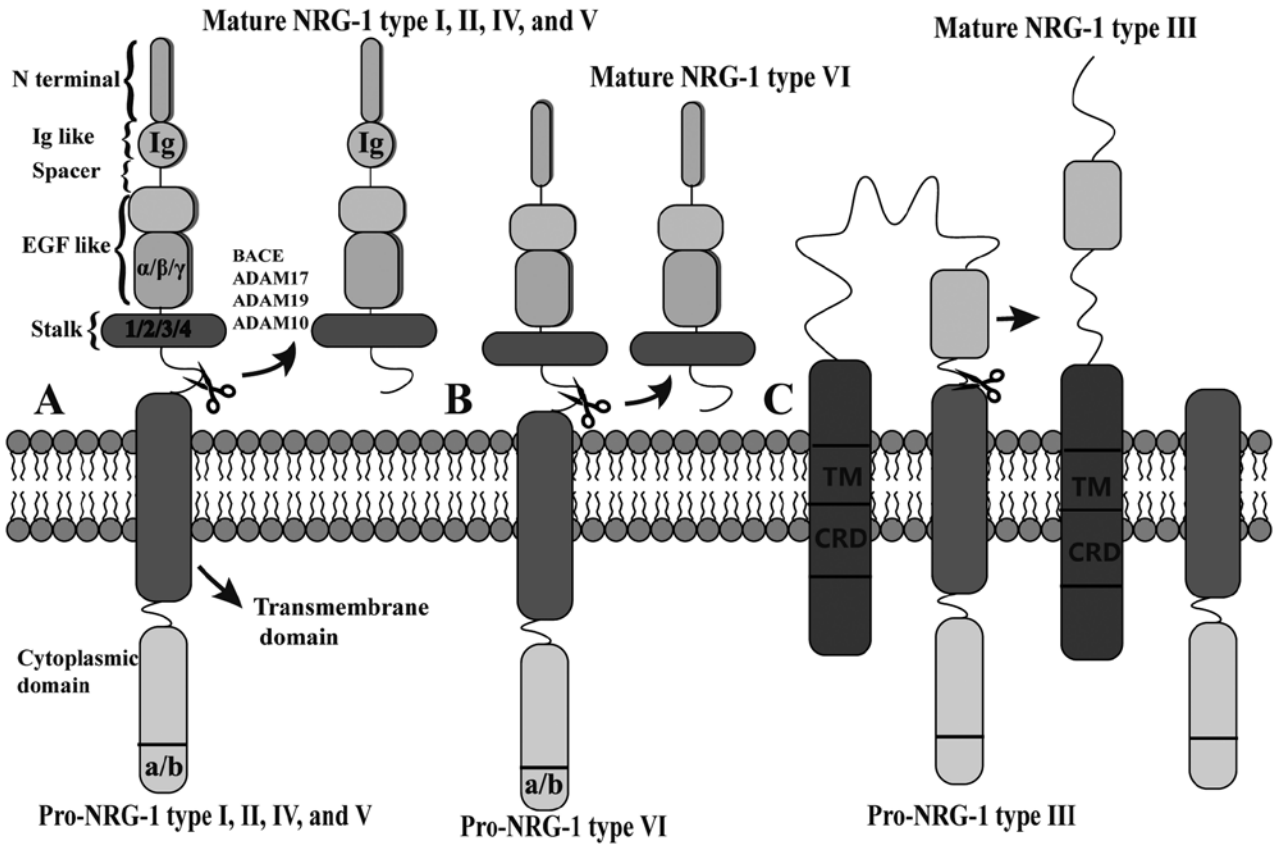

Figure 1. Structure of NRG-1 and characteristics of the 6 types. (A) NRG-1 types I, II, IV and V isoforms have an additional immunoglobulin (Ig)-like domain, while the (B) N-terminal region of types III and VI are connected directly to the EGF-like domain. Of note, (C) type III contains a cysteine-rich domain (CRD) and an additional N-terminal transmembrane domain (TM). Pro-NRG-1 undergoes type I transmembrane domain proteolysis (e.g., BACE, ADAM17, ADAM19 and ADAM10) to release the functional fragment, termed mature NRG-1, except in the case of NRG-1 type III. NRG-1, neuregulin-1.

and alternative/non-canonical pathways. The canonical NF-κB pathway involves the phosphorylation and degradation of inhibitory κB (IκB), resulting in the nuclear translocation of p65/p50 NF-κB heterodimers and ultimately triggering the production of pro-inflammatory molecules (31). By contrast, the alternative NF-κB pathway promotes the generation of anti-apoptotic and anti-inflammatory molecules through the enhanced translocation of RelB/p52 NF-κB heterodimers by the activation of IKB kinase-α (IKK-α) (32,33).

Studies have demonstrated the anti-inflammatory effects of NRG-1 in the setting of sepsis-induced cardiac injury and ischemic stroke (34,35). NRG-1 alleviates the inflammatory response by both the inhibition of canonical NF-κB and the activation of alternative NF-κB. Consistent with the alleviation of the inflammatory response, the pro-inflammatory factors [e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β] are downregulated and anti-inflammatory factors [e.g., granulocyte-colony stimulating factor (G-CSF) and IL-9] are upregulated (35). Furthermore, NRG-1/ErbB4 can inhibit the action of macrophages through the PI3K/AKT pathway, attenuating myocardium inflammation and fibrosis (36). Although there is limited evidence available to indicate that NRG-1 exerts its anti-inflammatory effects during IR to protect the heart, inflammation is a ubiquitous stress-response involving the entire body, and ischemic stroke shares similar features as myocardial infarction. Consequently, it can be inferred that NRG-1 modulates inflammation by regulating the NF-κB pathway and suppressing macrophage activation during IR injury, thus salvaging at-risk myocytes. On the other hand, repressing the maladaptive organelle response attributed to inflammation (e.g., oxidative stress, endoplasmic reticulum stress and calcium overload) may also be an approach to exert anti-inflammatory effects.

Endoplasmic reticulum (ER) stress. ER stress, as a transient adaptive response aimed at reducing the level of unfolded proteins and returning the ER to homeostasis, following the upregulation of molecular chaperones, ultimately increases the ability of the ER to regulate calcium content and protect myocardial cells (37,38). However, prolonged or severe ER stress, attributed to persistent hypoxia, oxidative stress and calcium overload, can abandon its pro-survival efforts and instead trigger apoptotic cell death by activating caspase-12 (an ER stress-specific proapoptotic molecule) and c-JUN N-terminal kinase and upregulating CCAAT/enhancer binding protein homologous protein (CHOP) (39). Increasing evidence has indicated that ER stress plays a crucial role in the pathogenesis of myocardial IR injury (40,41). It has been demonstrated that NRG-1/ErbB protects against cardiac IR injury by suppressing cardiac ER stress through the PI3K/Akt pathway and directly inhibits the upregulation of ER stress-related markers [e.g., glucose-regulated protein (GRP)78, CHOP and cleaved caspase-12] in both neonatal and adult ventricular myocytes to delay the onset of ER stress,

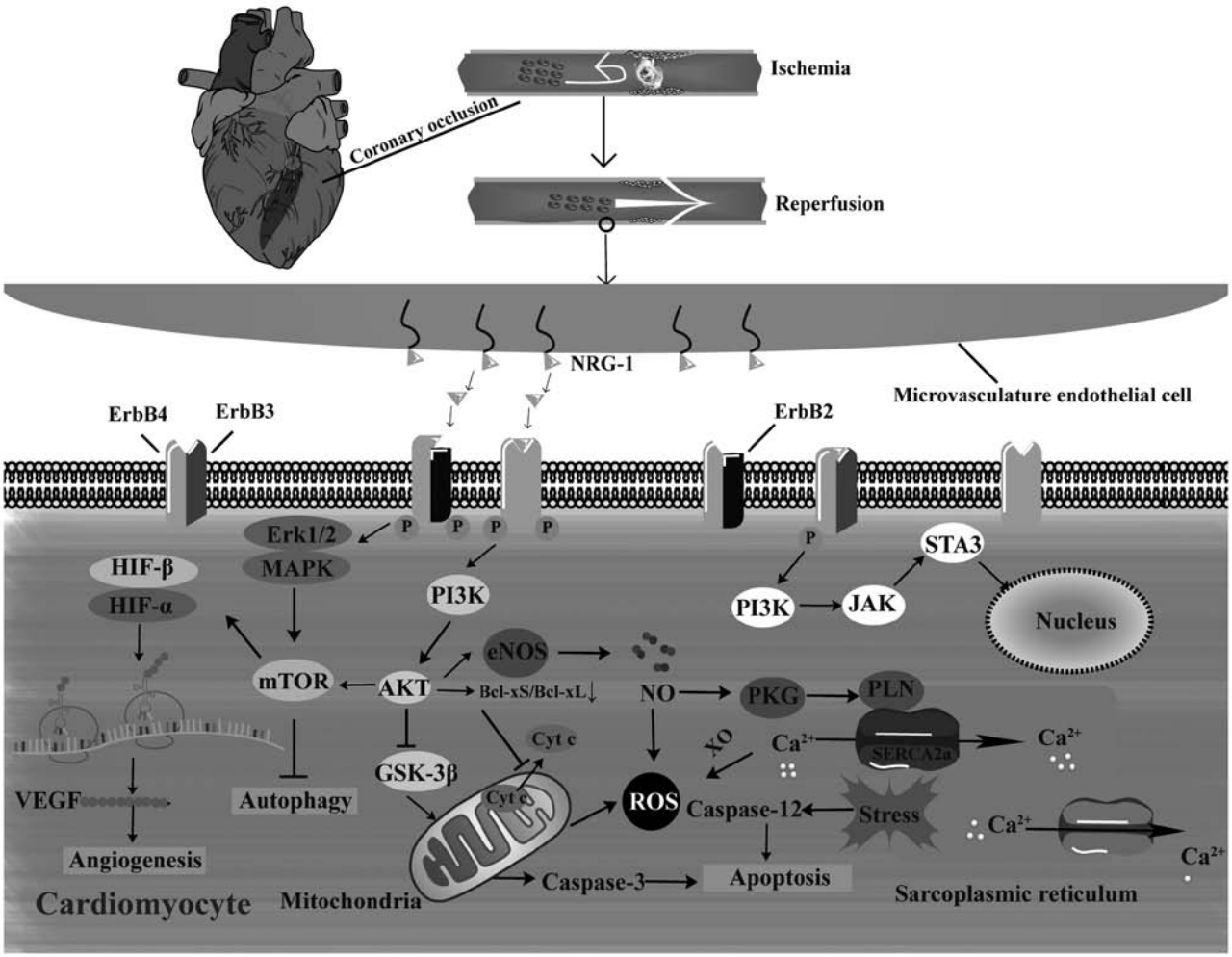

Figure 2. Mechanisms of action of NRG-1 in myocardial IR injury. NRG-1 is upregulated during myocardial IR injury and is released from the microvasculature endothelium by specific proteases. Upon ligand binding, NRG-1/ErbB activates several signaling pathways to protect against myocardial IR injury involved in endoplasmic reticulum stress, calcium overload, oxidative stress and cell death. The main signaling pathways include PI3K/Akt and Erk/MAPK, which are both important reperfusion injury salvage kinases. Cyt *c*, cytochrome; HIF, hypoxia-inducible factor; NO, nitric oxide; NRG-1, neuregulin-1; ROS, reactive oxygen species; SERCA2a, sarcoplasmic reticulum Ca^{2+}-ATPase; VEGF, vascular endothelial growth factor; XO, xanthine oxidase.

reducing caspase-12-related apoptosis (39). On the other hand, NRG-1 also hinders the onset of ER stress by alleviating oxidative stress and calcium overload, which will be discussed in detail below.

Calcium overload and nitric oxide (NO). The disequilibrium of intracellular Ca^{2+} homeostasis plays a significant role during the pathogenesis of IR injury and contributes to the impaired cardiac contractile function (3). ER stress-induced calcium overload is the upstream signal for IR injury (42). The increased level of calcium activates Ca^{2+}-dependent xanthine oxidase (XO), which results in ROS generation and cellular apoptosis (43).

NO, recognized as an inorganic free radical gas, is synthesized from the amino acid L-arginine by nitric oxide synthases (NOSs), such as neuronal NOS (nNOS or NOS I), inducible NOS (iNOS or NOS II), and endothelial NOS (eNOS or NOS III) (44). NO functions as an important biological molecule attenuating myocardial IR injury via the regulation of cardiac contractility and vasodilation (45). Under calcium overload, NRG-1 rapidly enhances the level of NO in adult ventricular myocytes through the activation of the PI3K/Akt/eNOS pathway. The increase in NO generation leads to PKG activation with the phosphorylation of phospholamban, which promotes sarcoplasmic reticulum calcium ATPase (SERCA2a) activity as well as calcium uptake by the sarcoplasmic reticulum (46). It has been shown that NRG-1 regulates calcium through the production of NO. The generation of NO also enhances the open probability of mitochondrial adenosine triphosphate-sensitive K^+ (mitoKATP) channels, but reduces the mPTP open probability. Notably, accumulated NO may exert a detrimental effect through the formation of peroxynitrite (a byproduct of NO degradation due to decreased NO bioavailability) (47), which aggravates oxidative stress and activates the apoptotic signaling pathway. It has been demonstrated that the hyperactivation of eNOS induced by NRG-1 can lead to an increase in ROS production (48), resulting in an increased MI size. The theory of eNOS uncoupling may explain this phenomenon. Briefly, under substrate (L-arginine) or cofactor deficiency, eNOS would transfer electrons to molecular oxygen rather than to substrate, resulting in the burst of ROS (49). It seems paradoxical due to the dual role of NO; however, its combination with L-arginine can reverse this deleterious effect.

Oxidative stress and HIF-1α. As previously mentioned, a surge of ROS has been shown to stimulate NRG-1 secretion. Moreover, NRG-1/ErbB can defend against oxidative stress during IR. Morano *et al* revealed that the expression of ErbB3 receptor in H9c2 cells increased the cell survival rate and enhanced mitochondrial resistance to oxidative stress by maintaining mitochondrial membrane potential (25), which inhibits the release of ROS and cytochrome *c* through the opening of mitoKATP channels. In addition to inhibiting the network of maladaptive organelle responses that aggravate oxidative stress, NRG-1 also induces adaptations of transcription factors involved in redox regulation, including 11 factors [catalase (CAT), Cu-Zn-dismutase (SOD1), thioredoxin (TXN), TXNRD1, protein disulfide-isomerase (PDI)A1, PDIA4, PDIA3, glutathione S-transferase Pi 1 (GSTP1), glutathione peroxidase 1 (GPX1), glutamate-cysteine ligase catalytic subunit (GCLC) and thiosulfate sulfurtransferase (TST)], to exert its radical scavenging effects (50). Furthermore, NRG-1 increases glutathione reductase mRNA, whose translation products are known as important antioxidants (51).

Hypoxia-inducible factor-1 (HIF-1), a heterodimeric transcription factor consisting of a constitutively expressed β subunit and an oxygen-regulated α subunit, regulates angiogenesis, proliferation and cellular metabolism, assisting cells in the adaption to hypoxic environments (52,53). Under anoxic conditions, HIF-1α becomes stable and is accompanied by an upregulated transcriptional response to ROS, inflammatory mediators and somatotropic hormone (54,55). Increased levels of HIF-1α and the enhanced transcriptional activity of key HIF-1 target genes, such as vascular endothelial growth factor (VEGF), play a critical role in myocardial IR injury. The activated HIF-1α/VEGF signaling pathway promotes the proliferation of cardiac microvasculature endothelial cells, which leads to NRG-1 generation and confers cardioprotective effects against anoxia injury during IR (56,57). It has previously been demonstrated that hypoxia can induce NRG-1 secretion and the phosphorylation of ErbB (27), and it has been shown that ErbB3 is upregulated by HIF-1α (58). Thus, it can be inferred that HIF-1α may upregulate NRG-1 via unidentified pathways. Of note, NRG-1 can in turn mediate HIF-1α expression. It has been demonstrated that the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway activated by NRG-1 can lead to HIF-1α activation and can regulate angiogenesis (59). Collectively, the interactions between HIF-1α and NRG-1 mediate angiogenesis by triggering an increase in the proliferation, migration and invasion of endothelial cells, as well as in the formation of a capillary-like tubular structure network. This helps cardiomyocytes to survive hypoxic conditions and under subsequent reperfusion injury.

Alleviation of cardiomyocyte death. The eventual and principal target of all therapeutic interventions is aimed at reducing the MI size and salvaging the myocardium. Thus, the prevention of cardiomyocyte death is crucial for cardiac function recovery and the estimation of intervention effects. Studies have demonstrated that the caspase-8-dependent Fas/FasL extrinsic death receptor pathway, caspase-9-related mitochondrial apoptosis and caspase-12-involved ER stress are associated with cardiomyocyte death in response to IR injury (60-62). Notably, NRG-1 has the ability to inhibit cell death by improving mitochondrial membrane potential (63), suppressing calcium overload (64), suppressing endoplasmic reticulum stress (38), alleviating the inflammatory response (35) and ultimately maintaining cellular viability during myocardial IR injury.

Apoptotic death. Apoptosis is an ATP-consuming form of programmed cell death characterized by chromatin accumulation, DNA fragmentation and apoptotic body formation, typically without an inflammatory response or membrane stability changes (2,65). Apoptosis can be initiated extrinsically by the activation of sarcolemma receptors (e.g., Fas and TNF-α) or intrinsically by the mitochondrial release of cytochrome *c*, which initiates a cascade of caspase activation and ultimately, intracellular proteolysis (66,67). NRG-1 has been demonstrated to directly suppress cardiomyocyte apoptosis by inhibiting mPTP opening, cytochrome *c* release and caspase-3 activation through PI3K/Akt signaling. Furthermore, the inhibition of ErbB2 and ErbB4 receptors leads to the induction of Bcl-xL splicing toward its pro-apoptotic protein Bcl-xS, thus activating mitochondrial dysfunction and apoptosis (68-70). This indirectly confirms the role of NRG-1/ErbB in myocyte apoptosis. Moreover, alleviating ER stress can inhibit the release of caspase-12, further reducing apoptotic death.

Autophagic death. Autophagy denotes a regulated process of lysosomal degradation and the recycling of cytoplasm or mitochondrial proteins (mitophagy), characterized by the formation of double-membrane vesicles (autophagosomes) and elevated levels of light chain 3, beclin-1, autophagy-related gene (ATG) 5-12 complex, p62 and parkin (71). It has been demonstrated that autophagy acts as a 'double-edged sword' in the pathology of IR injury. To the best of our knowledge, autophagy may exert beneficial effects during the early period of ischemia, but detrimental effects during the late period of ischemia and reperfusion (72). The present review focuses on the prevention of the detrimental effects induced by autophagy. It has been illustrated that the PI3K/PKB/mTOR and mitogen-activated protein kinase (MAPK)/ERK1/2/mTOR pathways activated by NRG-1/ErbB via phosphorylation of phosphatidylinositol are involved in the negative regulation of autophagy (73,74). Moreover, the Akt-mediated reduction of ROS (Akt/ROS signaling) results in the upregulation of Bcl-2, which plays a role in the anti-autophagy effects of NRG-1 (73).

4. Involvement of NRG-1 in conditioning against myocardial IR injury

Conditioning is a practice of applying brief episodes of intermittent nonlethal stimulus, which confers protection against myocardial IR injury (75). Considering the temporal association between the stimulus and ischemia, conditioning can be classified into 3 types, including preconditioning, perconditioning and postconditioning (76,77). More recently, some experimental studies have explored the association between NRG-1 and different types of conditioning (Table I). Although the mechanisms of NRG-1 involved in conditioning remain poorly understood, the present review focused on the NRG-1-induced cardioprotective effects against IR injury in terms of preconditioning and postconditioning (Fig. 3).

Table I. Involvement of NRG-1 in conditioning.

Author/(Refs.)	Year	Model	NRG-1 treatment	Timepoint	Species	Ischemia	Reperfusion
Fang *et al* (24)	2010	LAD ligature and reopening	4 μg/kg, IV	20 min before the hearts were subjected to IR	Rats	45 min	3 h
Ebner *et al* (49)	2013	LAD ligature and reopening	8 μg/kg, IP	5 min before reopening of the ligature	Mice	45 min	30 min
Wang *et al* (88)	2018	LAD ligature and reopening	3 μg/kg, IV	At the onset of reperfusion	Rats	45 min	24 h
		Langendorff isolated heart	20 ng/ml, perfused for 20 min	At the onset of reperfusion	Rats	30 min	2 h

NRG-1, neuregulin-1; LAD, left anterior descending artery; IV, intravenous injection; IP, intraperitoneal injection.

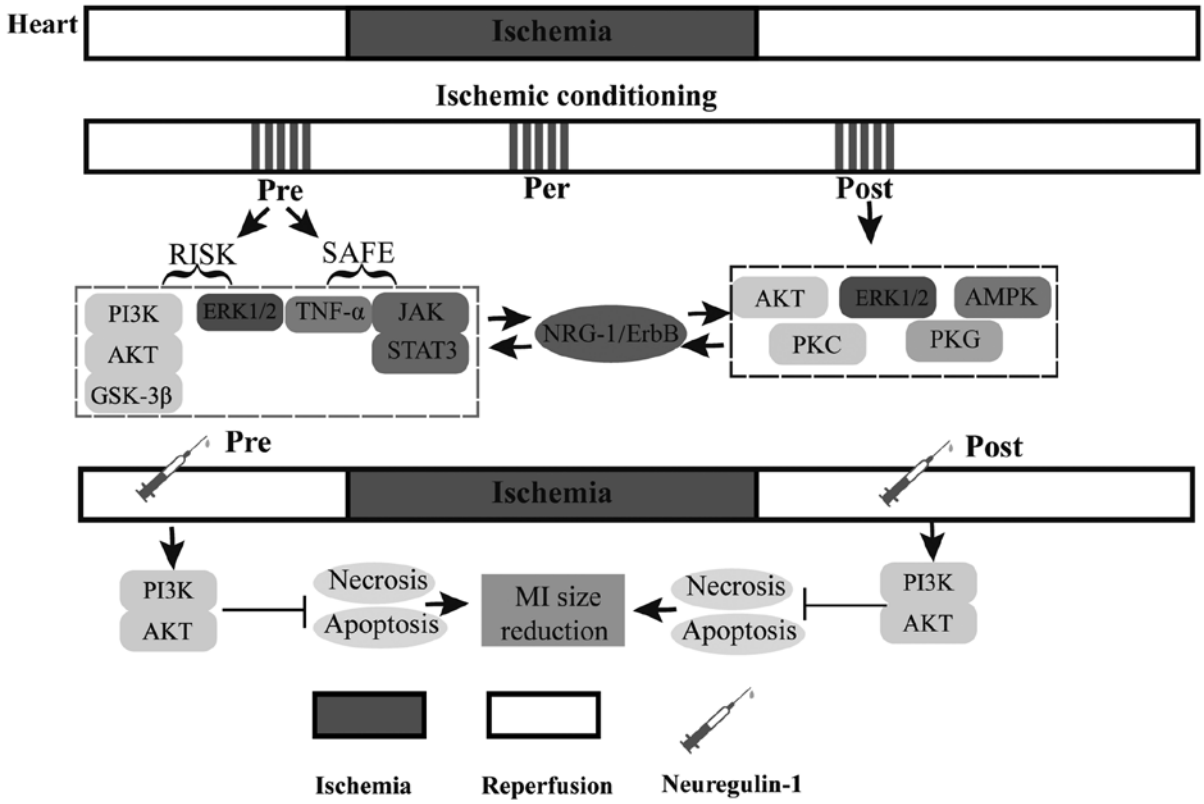

Figure 3. Potential association between conditioning and NRG-1. Ischemic conditioning (e.g., ischemic preconditioning and ischemic postconditioning) activate several signaling pathways that share common routes with the NRG-1/ErbB signaling axis. Preconditioning or postconditioning with neuregulin-1 reduces the MI size by repressing myocardium necrosis and apoptosis through the PI3K/AKT pathway. NRG-1, neuregulin-1; MI, myocardium infarction; RISK, reperfusion injury salvage kinase; SAFE, survivor activating factor enhancement.

Preconditioning. Preconditioning is the protective stimulus applied prior to the onset of a sustained episode of ischemia (78). Generally, preconditioning comprises two temporal windows, as well as protection peaks. The first window (acute form) occurs several minutes following the stimulus and lasts for 1-3 h, while the second window (delay form) begins a few hours (peak at 24 h) after the stimulus and lasts several days but no longer than 72 h (79). The major interventions of preconditioning include ischemic conditioning (IPC), pharmacological preconditioning, remote ischemic preconditioning (RIPC) and physical preconditioning, which have been shown to reduce the MI size (78,80). Hereinafter, the role of NRG-1 in IPC and pharmacological preconditioning is described.

IPC refers to a process of repetitive non-lethal ischemia prior to sustained lethal myocardial ischemia, which increases cardiac resistance against IR injury (81). It has been shown that the reperfusion injury salvage kinase [e.g., the PI3K/AKT/glycogen synthase kinase (GSK)-3β and ERK1/2 pathways] and the survivor activator factor enhancement pathways (e.g., TNF-α, JAK2/STAT3 pathway) are the main pathways involved in IPC-induced cardioprotection (75,82). Notably, NRG-1/ErbB shares the same signaling pathways with IPC as above, and

NRG-1 is rapidly upregulated during IPC and myocardial IR injury. This indicates that NRG-1 may at least partially play an important role in IPC-induced cardioprotection.

Pharmacological preconditioning is characterized by pre-treatment with drugs (78). *In vivo*, NRG-1 preconditioning protects the heart against IR injury by reducing myocardial necrosis and apoptosis mediated by a PI3K/Akt-dependent mechanism. Consistent with MI size limitations, NRG-1 preconditioning also decreases the level of plasma CK and LDH after 45 min of regional myocardial ischemia and 180 min of reperfusion (24). Thus, NRG-1 preconditioning is effective at reducing myocardium damage and represents a novel cardiac protective strategy that can be used in the setting of elective myocardial IR, as encountered during cardiac surgery and acute myocardial infarction.

Postconditioning. Postconditioning, defined as cardioprotective intervention applied at the onset of reperfusion following sustained ischemia (83), can be achieved by short repeated occlusions of the vessel prior to permanent reperfusion [ischemic postconditioning (IP)] or by pharmacological interventions [pharmacological postconditioning (pPC)], which both have recently been shown to have potential as novel cardioprotective interventions against IR injury (77).

For conferring protection against IR injury, IP plays a key role in the alleviation of oxidative stress, inflammation and apoptosis through NO production and mitoKATP channels opening by salvage kinase pathways, including AKT, ERK1/2, 5'AMP-activated protein kinase (AMPK), protein kinase (PK)C and PKG (84-86). Of note, *in vivo*, IP promotes NRG-1 protein expression, as well as the upregulation/activation of ErbB3 and ErbB4, indicating that the cardioprotection may be mediated by the NRG-1/ErbB3 and ErbB4 signaling pathways, which has also recently been confirmed in ischemic local postconditioning (87).

pPC with NRG-1 concurrently with reperfusion has been shown to inhibit apoptosis and reduce MI size in a IR rat model or isolated murine heart (25), exerting a cardioprotective effect via the PI3K/Akt pathway (87,88). Therefore, pPC with NRG-1 may be an effective treatment following timely reperfusion, such as thrombolytic therapy or primary percutaneous coronary intervention (PPCI).

5. Therapeutic potential of NRG-1 against myocardial IR injury

As accumulating evidence has indicated that the enhanced activation of the NRG-1/ErbB axis primarily contributes to attenuating myocardial IR injury, NRG-1 has emerged as a novel promising therapeutic alternative for reperfusion injury. Hereinafter, studies regarding other therapeutic potentials of NRG-1 in myocardial IR injury are mentioned and discussed.

Stem cell-based therapies. Recently, stem cell-based therapies have provided great promise for interventions in ischemic heart diseases (89,90). Among the forms of ischemic heart diseases, stem cells have been explored, particularly in the setting of myocardial infarction (91). In this connection, it has been reported that NRG-1 is a pivotal target of stem cell regulation, both in cultured cardiomyocytes and in whole embryos (5). In addition to mediating ventricular myocyte proliferation, NRG-1/ErbB has been found to be involved in embryonic stem cell (ESC) differentiation into cardiac myocyte lineages (92) and further, in the induction of cardiac conduction system cell differentiation. Moreover, the differentiation of stem cells into working-type cardiomyocytes can be modulated by NRG-1 (93). These effects can be interpreted as the upregulation of connexin with NRG-1 administration in ESC-derived cardiomyocytes, such as connexin 40 (Cx40) and connextin-45 (Cx45) (94). As intracellular channel proteins, connexins bridge gaps with cardiomyocytes to achieve synchronized contraction of the heart (95). The upregulated expression of connexins induced by NRG-1 also helps ESC differentiate into cardiac myocytes via MEK/ERK, and different cell lineages may be attributed to the different expression of connexin (94). It has been reported that treatment with cardiosphere-derived cells (CDCs) improves ventricular function in children with single ventricle physiology (96), which indicates the potential of cardiac self-repair. Since myocardial IR injury is inevitable in MI, further attention should be paid to restituting and restoring functional and structural components of the heart; stem cell transplantation administered with NRG-1 may be a strategic option.

Gene-based therapy. Gene-based therapy utilizes gene delivery systems (e.g., viral and non-viral vectors) to modulate gene expression at the cellular level to treat pathological conditions (97). As regards IR injury, it has been reported that lentivirus-mediated hNRG-1 gene transduction establishes a stable expression system in infarcted hearts of rats and further activates the PI3K/Akt/eNOS pathway to promote neovascularization and angiogenesis, as manifested by enhanced expression of VEGF. In addition, the overexpression of hNRG-1 alleviates myocyte apoptosis through the Bcl-2/Bax signaling pathway (98). Collectively, the gene-based therapy of NRG-1 helps attenuate IR injury and eventually improves cardiac function. Although the application is still limited to animal experiments, and gene-based therapy has not yet been popularized, the significant protective effects suggest that gene delivery can be an alternative approach for NRG-1-dependent therapeutic strategies against myocardial IR injury.

NRG-1-loaded microparticles. The widespread clinical use of cardiovascular protein treatment may be hampered due to the limited stability and rapid degradation of protein, and novel formulation strategies that take into account sustained drug bioavailability in the infarcted border zone are urgently required (99,100). The application of microparticles (MPs) through catheter-based intramyocardial injection, with minimally invasive methods, may be a desirable approach for the clinical translation of cardiac regenerative medicine of MI (101). Briefly, cardiovascular protein molecules, such as NRG-1, are encapsulated into delicate bioresorbable scaffolds (PLGA) to form MPs and injected into target cardiac tissue with the guidance of visual cardiac mapping to achieve precise treatment (102). It has previously been demonstrated that NRG-1 plays critical roles in cardiac remodeling and MI size limitation through RISK and survivor activating factor enhancement (SAFE) pathways (103), and these benefits can be maximally utilized in the target infarcted

zone. NRG-1-loaded MPs have been previously applied in a porcine model of IR over a period of months without severe side-effects; additionally, a prolonged and effective angiogenic stimulus was provided to the ischemic myocardium due to the sustained release, which failed to achieve success in clinical trials by applying pro-angiogenic factors (104). Notably, the transplantation of adipose-derived stem cells combined with NRG-1-loaded MPs stimulated cardiomyocyte proliferation and provided more complete healing in a rat myocardial infarction (105), suggesting that 'the whole is greater than the sum of its parts.' With the growing morbidity of AMI and the progress being made in precision medicine, NRG-1-loaded MPs may be a promising treatment for patients with MI by enhancing patient compliance and curative effects.

Cardiac transplantation. Cardiac transplantation, considered as the only effective therapy for end-stage heart failure, requires appropriate storage conditions for donor hearts to attenuate IR injury and preserve heart function during reperfusion (106). It has been demonstrated that rhNRG-1 mitigates left ventricular remodeling and sarcomere disorganization by upregulation of the RISK pathway (107,108). Notably, by combining organ-storage solution (Celsior) with rhNRG-1 in an isolated working rat heart model, additional cardiac preservation was observed after hypothermic storage, as evidenced by the reduction of myocyte apoptosis and necrosis during transplantation. Moreover, this recovery function could be enhanced by combination with other cardioprotective agents (e.g., glyceryl trinitrate and cariporide) (109). Accompanied by increased steady-state level of phosphorylated kinases [e.g., p-Akt, p-ERK1/2, p-signal transducer and activator of transcription 3 (STAT3) and p-GSK-3β] and reduced cleaved caspase-3, NRG-1 may exert these benefits by activating downstream pathways, including Akt, Ekr1/2 and JAK/STAT3, and involving caspase-3 related apoptosis (109,110). With rhNRG-1 supplementation, the goals of a longer storage time and higher cardiac vitality can be achieved during transplantation, and the success rate of surgery can be enhanced due to attenuation of IR injury. This suggests that NRG-1 may partially mitigate the contradiction between wanting donor hearts and growing clinical needs via the potential benefit against myocardial IR injury.

6. Conclusion and future perspectives

Recently, increasing evidence has gradually revealed the potential cardiac benefits of NRG-1 against myocardial IR injury. In this regard, it has been demonstrated that NRG-1 modulates several endocellular transcripts (e.g., SOD1 and TXN), important mediators (e.g., NO and HIF-1α) and signaling pathways (e.g., PI3K/Akt and MAPK/ERK1/2 pathways), thereby forming a complicated network that contributes to straining the inflammatory response, alleviating ER stress, suppressing calcium overload, inhibiting oxidative stress and repressing cellular death (e.g., apoptosis and autophagy) in cardiomyocytes during IR injury.

Endogenous NRG-1 is a potential cardioprotective mediator of conditioning, and preconditioning or postconditioning with exogenous NRG-1 also confers cardioprotective effects against IR injury. However, the mechanisms underlying the involvement of NRG-1 in conditioning are not yet clear and warrant more in-depth investigation. Significantly, several therapeutic potentials of NRG-1 have been revealed (e.g., the application of NRG-1 in stem cell-based therapies, gene transduction, microparticle delivery of hNRG-1 and cardiac transplantation), which may assist in expanding the therapeutic strategies of NRG-1 against myocardial IR injury in clinical practice.

Finally, for future research perspectives, the further directions of NRG-1 research in myocardial IR are as follows: i) NRG-1 promotes glucose uptake independently of insulin in the liver and cardiomyocytes via the PI3Kα/Akt/AS160 pathway and GLUT4 translocation (111,112), which illuminates possible research of diabetic patients with acute coronary syndrome, such as the application of neuregulin-1 in myocardial IR rats with diabetes; ii) the neuregulin-1/ErbB pathway enhances leptin levels and improves behavior against obesity, enlightening the possible approach of exploring underlying the mechanisms of action of NRG-1 in a myocardial IR model with obesity or a high-fat diet (113,114); iii) crosstalk between NRG-1 and HIF-1 remains poorly understood and warrants further in-depth exploration, such as the level of change of HIF-1 in hypoxia/reoxygenation (H/R) cardiomyocytes accompanied by NRG-1 treatment.

In conclusion, the NRG-1/ErbB network is a critical modulator of IR injury, and NRG-1 may be a promising therapeutic target in the future.

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Authors' contributions

YL reviewed the related science literature and wrote the manuscript. HL supported YL in the revisions of the manuscript and processing of the figures. XW and HL conceived the study and supervised the writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that there are no competing interests.

References

1. Chan SH, Hung CH, Shih JY, Chu PM, Cheng YH, Lin HC and Tsai KL: SIRT1 inhibition causes oxidative stress and inflammation in patients with coronary artery disease. Redox Biol 13: 301-309, 2017.
2. Ibáñez B, Heusch G, Ovize M and Van de Werf F: Evolving therapies for myocardial ischemia/reperfusion injury. J Am Coll Cardiol 65: 1454-1471, 2015.
3. Zhou H, Ma Q, Zhu P, Ren J, Reiter RJ and Chen Y: Protective role of melatonin in cardiac ischemia-reperfusion injury: From pathogenesis to targeted therapy. J Pineal Res 64, 2018.
4. Mendes-Ferreira P, De Keulenaer GW, Leite-Moreira AF and Brás-Silva C: Therapeutic potential of neuregulin-1 in cardiovascular disease. Drug Discov Today 18: 836-842, 2013.
5. Rupert CE and Coulombe KL: The roles of neuregulin-1 in cardiac development, homeostasis, and disease. Biomarker Insights 10 (Suppl 1): S1-S9, 2015.
6. Liu YQ, Yang M, Duan CH, Su GB, Wang JH, Liu YF and Zhang J: Protective role of neuregulin-1 toward doxorubicin-induced myocardial toxicity. Genet Mol Res 13: 4627-4634, 2014.
7. Galindo CL, Kasasbeh E, Murphy A, Ryzhov S, Lenihan S, Ahmad FA, Williams P, Nunnally A, Adcock J, Song Y, *et al*: Anti-remodeling and anti-fibrotic effects of the neuregulin-1beta glial growth factor 2 in a large animal model of heart failure. J Am Heart Assoc 3: e000773, 2014.
8. Miao J, Huang S, Su YR, Lenneman CA, Wright M, Harrell FE, Sawyer DB and Lenihan DJ: Effects of endogenous serum neuregulin-1β on morbidity and mortality in patients with heart failure and left ventricular systolic dysfunction. Biomarkers 23: 704-708, 2018.
9. Chou CF and Ozaki M: In silico analysis of neuregulin 1 evolution in vertebrates. Biosci Rep 30: 267-275, 2010.
10. Kataria H, Alizadeh A and Karimi-Abdolrezaee S: Neuregulin-1/ErbB network: An emerging modulator of nervous system injury and repair. Prog Neurobiol 180: 101643, 2019.
11. Liu X, Bates R, Yin DM, Shen C, Wang F, Su N, Kirov SA, Luo Y, Wang JZ, Xiong WC and Mei L: Specific regulation of NRG1 isoform expression by neuronal activity. J Neurosci 31: 8491-8501, 2011.
12. Tan W, Wang Y, Gold B, Chen J, Dean M, Harrison PJ, Weinberger DR and Law AJ: Molecular cloning of a brain-specific, developmentally regulated neuregulin 1 (NRG1) isoform and identification of a functional promoter variant associated with schizophrenia. J Biol Chem 282: 24343-24351, 2007.
13. Hu X, Fan Q, Hou H and Yan R: Neurological dysfunctions associated with altered BACE1-dependent Neuregulin-1 signaling. J Neurochem 136: 234-249, 2016.
14. Zhang Z, Huang J, Shen Y and Li R: BACE1-dependent Neuregulin-1 signaling: An implication for schizophrenia. Front Mol Neurosci 10: 302, 2017.
15. Willem M: Proteolytic processing of Neuregulin-1. Brain Res Bull 126: 178-182, 2016.
16. Mostaid MS, Lloyd D, Liberg B, Sundram S, Pereira A, Pantelis C, Karl T, Weickert CS, Everall IP and Bousman CA: Neuregulin-1 and schizophrenia in the genome-wide association study era. Neurosci Biobehav Rev 68: 387-409, 2016.
17. Nagano T, Namba H, Abe Y, Aoki H, Takei N and Nawa H: In vivo administration of epidermal growth factor and its homologue attenuates developmental maturation of functional excitatory synapses in cortical GABAergic neurons. Eur J Neurosci 25: 380-390, 2007.
18. Olayioye MA, Neve RM, Lane HA and Hynes NE: The ErbB signaling network: Receptor heterodimerization in development and cancer. EMBO J 19: 3159-3167, 2000.
19. Schlessinger J: Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell 110: 669-672, 2002.
20. D'Uva G and Lauriola M: Towards the emerging crosstalk: ERBB family and steroid hormones. Semin Cell Dev Biol 50: 143-152, 2016.
21. Li KX, Lu YM, Xu ZH, Zhang J, Zhu JM, Zhang JM, Cao SX, Chen XJ, Chen Z, Luo JH, *et al*: Neuregulin 1 regulates excitability of fast-spiking neurons through Kv1.1 and acts in epilepsy. Nat Neurosci 15: 267-273, 2011.
22. Falls D: Neuregulins: Functions, forms, and signaling strategies. Exp Cell Res 284: 14-30, 2003.
23. Odiete O, Hill MF and Sawyer DB: Neuregulin in cardiovascular development and disease. Circ Res 111: 1376-1385, 2012.
24. Fang SJ, Wu XS, Han ZH, Zhang XX, Wang CM, Li XY, Lu LQ and Zhang JL: Neuregulin-1 preconditioning protects the heart against ischemia/reperfusion injury through a PI3K/Akt-dependent mechanism. Chin Med J (Engl) 123: 3597-3604, 2010.
25. Morano M, Angotti C, Tullio F, Gambarotta G, Penna C, Pagliaro P and Geuna S: Myocardial ischemia/reperfusion upregulates the transcription of the Neuregulin1 receptor ErbB3, but only postconditioning preserves protein translation: Role in oxidative stress. Int J Cardiol 233: 73-79, 2017.
26. Kuramochi Y, Cote GM, Guo X, Lebrasseur NK, Cui L, Liao R and Sawyer DB: Cardiac endothelial cells regulate reactive oxygen species-induced cardiomyocyte apoptosis through neuregulin-1beta/erbB4 signaling. J Biol Chem 279: 51141-51147, 2004.
27. Iivanainen E, Paatero I, Heikkinen SM, Junttila TT, Cao R, Klint P, Jaakkola PM, Cao Y and Elenius K: Intra- and extracellular signaling by endothelial neuregulin-1. Exp Cell Res 313: 2896-2909, 2007.
28. Griffiths HR, Gao D and Pararasa C: Redox regulation in metabolic programming and inflammation. Redox Biol 12: 50-57, 2017.
29. Nahrendorf M, Pittet MJ and Swirski FK: Monocytes: Protagonists of infarct inflammation and repair after myocardial infarction. Circulation 121: 2437-2445, 2010.
30. Vilahur G and Badimon L: Ischemia/reperfusion activates myocardial innate immune response: The key role of the toll-like receptor. Front Physiol 5: 496, 2014.
31. Hoesel B and Schmid JA: The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 12: 86, 2013.
32. Sun SC: Non-canonical NF-κB signaling pathway. Cell Res 21: 71-85, 2011.
33. Sun SC: The non-canonical NF-κB pathway in immunity and inflammation. Nat Rev Immunol 17: 545-558, 2017.
34. Zhou Q, Wang L, Wang X, Xiong Q, Wei Y, Dang S and Zhong L: Effect of neuregulin-1 on heart function and inflammatory mediators in rats with sepsis. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 30: 140-144, 2018 (In Chinese).
35. Simmons LJ, Surles-Zeigler MC, Li Y, Ford GD, Newman GD and Ford BD: Regulation of inflammatory responses by neuregulin-1 in brain ischemia and microglial cells in vitro involves the NF-kappa B pathway. J Neuroinflammation 13: 237, 2016.
36. Vermeulen Z, Hervent AS, Dugaucquier L, Vandekerckhove L, Rombouts M, Beyens M, Schrijvers DM, De Meyer GRY, Maudsley S, De Keulenaer GW and Segers VFM: Inhibitory actions of the NRG-1/ErbB4 pathway in macrophages during tissue fibrosis in the heart, skin, and lung. Am J Physiol Heart Circ Physiol 313: H934-H945, 2017.
37. Liu MQ, Chen Z and Chen LX: Endoplasmic reticulum stress: A novel mechanism and therapeutic target for cardiovascular diseases. Acta Pharmacol Sin 37: 425-443, 2016.
38. Xu M, Wu X, Jie B, Zhang X, Zhang J, Xin Y and Guo Y: Neuregulin-1 protects myocardial cells against H_2O_2-induced apoptosis by regulating endoplasmic reticulum stress. Cell biochemistry and function 32: 464-469, 2014.
39. Fang SJ, Li PY, Wang CM, Xin Y, Lu WW, Zhang XX, Zuo S, Ma CS, Tang CS, Nie SP and Qi YF: Inhibition of endoplasmic reticulum stress by neuregulin-1 protects against myocardial ischemia/reperfusion injury. Peptides 88: 196-207, 2017.
40. Groenendyk J, Agellon LB and Michalak M: Coping with endoplasmic reticulum stress in the cardiovascular system. Annu Rev Physiol 75: 49-67, 2013.
41. Wu H, Ye M, Yang J, Ding J, Yang J, Dong W and Wang X: Nicorandil protects the heart from ischemia/reperfusion injury by attenuating endoplasmic reticulum response-induced apoptosis through PI3K/Akt signaling pathway. Cell Physiol Biochem 35: 2320-2332, 2015.
42. Zhu H, Jin Q, Li Y, Ma Q, Wang J, Li D, Zhou H and Chen Y: Melatonin protected cardiac microvascular endothelial cells against oxidative stress injury via suppression of IP3R-[Ca^{2+}]c/VDAC-[Ca^{2+}]m axis by activation of MAPK/ERK signaling pathway. Cell Stress Chaperones 23: 101-113, 2018.
43. Zhang Y, Zhou H, Wu W, Shi C, Hu S, Yin T, Ma Q, Han T, Zhang Y, Tian F and Chen Y: Liraglutide protects cardiac microvascular endothelial cells against hypoxia/reoxygenation injury through the suppression of the SR-Ca(2+)-XO-ROS axis via activation of the GLP-1R/PI3K/Akt/survivin pathways. Free Radic Biol Med 95: 278-292, 2016.
44. Förstermann U and Sessa WC: Nitric oxide synthases: Regulation and function. Eur Heart J 33: 829-837, 837a-837d, 2012.

45. Yu X, Ge L, Niu L, Lian X, Ma H and Pang L: The dual role of inducible nitric oxide synthase in myocardial ischemia/reperfusion injury: Friend or foe? Oxid Med Cell Longev 2018: 8364848, 2018.
46. Brero A, Ramella R, Fitou A, Dati C, Alloatti G, Gallo MP and Levi R: Neuregulin-1beta1 rapidly modulates nitric oxide synthesis and calcium handling in rat cardiomyocytes. Cardiovasc Research 88: 443-452, 2010.
47. Cadenas S: ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. Free Radic Biol Med 117: 76-89, 2018.
48. Lemmens K, Fransen P, Sys SU, Brutsaert DL and De Keulenaer GW: Neuregulin-1 induces a negative inotropic effect in cardiac muscle: Role of nitric oxide synthase. Circulation 109: 324-326, 2004.
49. Ebner B, Lange SA, Eckert T, Wischniowski C, Ebner A, Braun-Dullaeus RC, Weinbrenner C, Wunderlich C, Simonis G and Strasser RH: Uncoupled eNOS annihilates neuregulin-1β-induced cardioprotection: A novel mechanism in pharmacological postconditioning in myocardial infarction. Mol Cell Biochem 373: 115-123, 2013.
50. Giraud MN, Fluck M, Zuppinger C and Suter TM: Expressional reprogramming of survival pathways in rat cardiocytes by neuregulin-1beta. J Appl Physiol (1985) 99: 313-322, 2005.
51. Timolati F, Ott D, Pentassuglia L, Giraud MN, Perriard JC, Suter TM and Zuppinger C: Neuregulin-1 beta attenuates doxorubicin-induced alterations of excitation-contraction coupling and reduces oxidative stress in adult rat cardiomyocytes. J Mol Cell Cardiol 41: 845-854, 2006.
52. Greer SN, Metcalf JL, Wang Y and Ohh M: The updated biology of hypoxia-inducible factor. EMBO J 31: 2448-2460, 2012.
53. Zimna A and Kurpisz M: Hypoxia-inducible factor-1 in physiological and pathophysiological angiogenesis: Applications and therapies. Biomed Res Int 2015: 549412, 2015.
54. Wang W, Xu B, Xuan H, Ge Y, Wang Y, Wang L, Huang J, Fu W, Michie SA and Dalman R: Hypoxia-inducible factor 1 in clinical and experimental aortic aneurysm disease. J Vasc Surg 68: 1538-1550.e2, 2018.
55. Movafagh S, Crook S and Vo K: Regulation of hypoxia-inducible factor-1a by reactive oxygen species: New developments in an old debate. J Cell Biochem 116: 696-703, 2015.
56. Wang J, Zhou J, Wang Y, Yang C, Fu M, Zhang J, Han X, Li Z, Hu K and Ge J: Qiliqiangxin protects against anoxic injury in cardiac microvascular endothelial cells via NRG-1/ErbB-PI3K/Akt/mTOR pathway. J Cell Mol Med 21: 1905-1914, 2017.
57. Lim CS, Kiriakidis S, Sandison A, Paleolog EM and Davies AH: Hypoxia-inducible factor pathway and diseases of the vascular wall. J Vasc Surg 58: 219-230, 2013.
58. Humtsoe JO, Pham E, Louie RJ, Chan DA and Kramer RH: ErbB3 upregulation by the HNSCC 3D microenvironment modulates cell survival and growth. Oncogene 35: 1554-1564, 2016.
59. Karar J and Maity A: PI3K/AKT/mTOR pathway in angiogenesis. Front Mol Neurosci 4: 51, 2011.
60. Kozlov AV, Lancaster JR Jr, Meszaros AT and Weidinger A: Mitochondria-meditated pathways of organ failure upon inflammation. Redox Biol 13: 170-181, 2017.
61. Liu XM, Yang ZM and Liu XK: Fas/FasL induces myocardial cell apoptosis in myocardial ischemia-reperfusion rat model. Eur Rev Med Pharmaco 21: 2913-2918, 2017.
62. Groenendyk J, Sreenivasaiah PK, Kim DH, Agellon LB and Michalak M: Biology of endoplasmic reticulum stress in the heart. Circ Res 107: 1185-1197, 2010.
63. Zhang XX, Wu XS, Mi SH, Fang SJ, Liu S, Xin Y and Zhao QM: Neuregulin-1 promotes mitochondrial biogenesis, attenuates mitochondrial dysfunction, and prevents hypoxia/reoxygenation injury in neonatal cardiomyocytes. Cell Biochem Funct: Feb 10, 2020 (Epub ahead of print).
64. Wang X, Zhuo X, Gao J, Liu H, Lin F and Ma A: Neuregulin-1β partially improves cardiac function in volume-overload heart failure through regulation of abnormal calcium handling. Front Pharmacol 10: 616, 2019.
65. Badalzadeh R, Mokhtari B and Yavari R: Contribution of apoptosis in myocardial reperfusion injury and loss of cardioprotection in diabetes mellitus. J Physiol Sci 65: 201-215, 2015.
66. Orogo AM and Gustafsson AB: Cell death in the myocardium: My heart won't go on. IUBMB life 65: 651-656, 2013.
67. Kleinbongard P, Schulz R and Heusch G: TNFα in myocardial ischemia/reperfusion, remodeling and heart failure. Heart Fail Rev 16: 49-69, 2011.
68. Rohrbach S, Muller-Werdan U, Werdan K, Koch S, Gellerich NF and Holtz J: Apoptosis-modulating interaction of the neuregulin/erbB pathway with anthracyclines in regulating Bcl-xS and Bcl-xL in cardiomyocytes. J Mol Cell Cardiol 38: 485-493, 2005.
69. Kuramochi Y, Lim CC, Guo X, Colucci WS, Liao R and Sawyer DB: Myocyte contractile activity modulates norepinephrine cytotoxicity and survival effects of neuregulin-1beta. Am J Physiol Cell Physiol 286: C222-C229, 2004.
70. Fukazawa R, Miller TA, Kuramochi Y, Frantz S, Kim YD, Marchionni MA, Kelly RA and Sawyer DB: Neuregulin-1 protects ventricular myocytes from anthracycline-induced apoptosis via erbB4-dependent activation of PI3-kinase/Akt. J Mol Cell Cardiol 35: 1473-1479, 2003.
71. Dong Y, Undyala VV, Gottlieb RA, Mentzer RM Jr and Przyklenk K: Autophagy: Definition, molecular machinery, and potential role in myocardial ischemia-reperfusion injury. J Cardiovasc Pharmacol Ther 15: 220-230, 2010.
72. Aghaei M, Motallebnezhad M, Ghorghanlu S, Jabbari A, Enayati A, Rajaei M, Pourabouk M, Moradi A, Alizadeh AM and Khori V: Targeting autophagy in cardiac ischemia/reperfusion injury: A novel therapeutic strategy. J Cell Physiol 234: 16768-16778, 2019.
73. An T, Huang Y, Zhou Q, Wei BQ, Zhang RC, Yin SJ, Zou CH, Zhang YH and Zhang J: Neuregulin-1 attenuates doxorubicin-induced autophagy in neonatal rat cardiomyocytes. J Cardiovasc Pharmacol 62: 130-137, 2013.
74. Surviladze Z, Sterk RT, DeHaro SA and Ozbun MA: Cellular entry of human papillomavirus type 16 involves activation of the phosphatidylinositol 3-kinase/Akt/mTOR pathway and inhibition of autophagy. J Virol 87: 2508-2517, 2013.
75. Sanada S, Komuro I and Kitakaze M: Pathophysiology of myocardial reperfusion injury: Preconditioning, postconditioning, and translational aspects of protective measures. Am J Physiol Heart Circ Physiol 301: H1723-H1741, 2011.
76. Hausenloy DJ and Yellon DM: The therapeutic potential of ischemic conditioning: An update. Nat Rev Cardiol 8: 619-629, 2011.
77. Heusch G: Treatment of myocardial ischemia/reperfusion injury by ischemic and pharmacological postconditioning. Compr Physiol 5: 1123-1145, 2015.
78. Hausenloy DJ and Yellon DM: Ischaemic conditioning and reperfusion injury. Nat Rev Cardiol 13: 193-209, 2016.
79. Heusch G: Molecular basis of cardioprotection: Signal transduction in ischemic pre-, post-, and remote conditioning. Circ Res 116: 674-699, 2015.
80. Stokfisz K, Ledakowicz-Polak A, Zagorski M and Zielinska M: Ischaemic preconditioning-current knowledge and potential future applications after 30 years of experience. Adv Med Sci 62: 307-316, 2017.
81. Diaz RJ and Wilson GJ: Studying ischemic preconditioning in isolated cardiomyocyte models. Cardiovasc Res 70: 286-296, 2006.
82. Rossello X and Yellon DM: The RISK pathway and beyond. Basic Res Cardiol 113: 2, 2018.
83. Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D, Hausenloy DJ, Heusch G, Vinten-Johansen J, Yellon DM and Schulz R; Working Group of Cellular Biology of Heart of European Society of Cardiology: Postconditioning and protection from reperfusion injury: Where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res 87: 406-423, 2010.
84. Hao M, Zhu S, Hu L, Zhu H, Wu X and Li Q: Myocardial ischemic postconditioning promotes autophagy against ischemia reperfusion injury via the activation of the nNOS/AMPK/mTOR pathway. Int J Mol Sci 18: 614, 2017.
85. Jivraj N, Liew F and Marber M: Ischaemic postconditioning: Cardiac protection after the event. Anaesthesia 70: 598-612, 2015.
86. Bice JS and Baxter GF: Postconditioning signalling in the heart: Mechanisms and translatability. Br J Pharmacol 172: 1933-1946, 2015.
87. Pilz PM, Hamza O, Gidlöf O, Gonçalves IF, Tretter EV, Trojanek S, Abraham D, Heber S, Haller PM, Podesser BK and Kiss A: Remote ischemic perconditioning attenuates adverse cardiac remodeling and preserves left ventricular function in a rat model of reperfused myocardial infarction. Int J Cardiol 285: 72-79, 2019.
88. Wang F, Wang H, Liu X, Yu H, Zuo B, Song Z, Wang N, Huang W and Wang G: Pharmacological postconditioning with Neuregulin-1 mimics the cardioprotective effects of ischaemic postconditioning via ErbB4-dependent activation of reperfusion injury salvage kinase pathway. Mol Med 24: 39, 2018.

89. Khanabdali R, Rosdah AA, Dusting GJ and Lim SY: Harnessing the secretome of cardiac stem cells as therapy for ischemic heart disease. Biochem Pharmacol 113: 1-11, 2016.
90. Yu H, Lu K, Zhu J and Wang J: Stem cell therapy for ischemic heart diseases. Br Med Bull 121: 135-154, 2017.
91. Barzegar M, Kaur G, Gavins FNE, Wang Y, Boyer CJ and Alexander JS: Potential therapeutic roles of stem cells in ischemia-reperfusion injury. Stem Cell Res 37: 101421, 2019.
92. Sun M, Yan X, Bian Y, Caggiano AO and Morgan JP: Improving murine embryonic stem cell differentiation into cardiomyocytes with neuregulin-1: Differential expression of microRNA. Am J Physiol Cell Physiol 301: C21-C30, 2011.
93. Zhu WZ, Xie Y, Moyes KW, Gold JD, Askari B and Laflamme MA: Neuregulin/ErbB signaling regulates cardiac subtype specification in differentiating human embryonic stem cells. Circ Res 107: 776-786, 2010.
94. Wang Z and Huang J: Neuregulin-1 increases connexin-40 and connexin-45 expression in embryonic stem cell-derived cardiomyocytes. Appl Biochem Biotechnol 174: 483-493, 2014.
95. Schulz R, Gorge PM, Gorbe A, Ferdinandy P, Lampe PD and Leybaert L: Connexin 43 is an emerging therapeutic target in ischemia/reperfusion injury, cardioprotection and neuroprotection. Pharmacol Ther 153: 90-106, 2015.
96. Ummarino D: Heart failure: Recombinant neuregulin for HF treatment. Nat Rev Cardiol 14: 128, 2017.
97. Cao Y, Tan YF, Wong YS, Liew MWJ and Venkatraman S: Recent advances in chitosan-based carriers for gene delivery. Mar Drugs 17: 381, 2019.
98. Xiao J, Li B, Zheng Z, Wang M, Peng J, Li Y and Li Z: Therapeutic effects of neuregulin-1 gene transduction in rats with myocardial infarction. Coron Artery Dis 23: 460-468, 2012.
99. Xiao S and Shaw RM: Cardiomyocyte protein trafficking: Relevance to heart disease and opportunities for therapeutic intervention. Trends Cardiovasc Med 25: 379-389, 2015.
100. Wang ZV and Hill JA: Protein quality control and metabolism: Bidirectional control in the heart. Cell Metab 21: 215-226, 2015.
101. Pascual-Gil S, Abizanda G, Iglesias E, Garbayo E, Prósper F and Blanco-Prieto MJ: NRG1 PLGA MP locally induce macrophage polarisation toward a regenerative phenotype in the heart after acute myocardial infarction. J Drug Target 27: 573-581, 2019.
102. Pascual-Gil S, Simon-Yarza T, Garbayo E, Prosper F and Blanco-Prieto MJ: Cytokine-loaded PLGA and PEG-PLGA microparticles showed similar heart regeneration in a rat myocardial infarction model. Int J Pharm 523: 531-533, 2017.
103. Kirabo A, Ryzhov S, Gupte M, Sengsayadeth S, Gumina RJ, Sawyer DB and Galindo CL: Neuregulin-1β induces proliferation, survival and paracrine signaling in normal human cardiac ventricular fibroblasts. J Mol Cell Cardiol 105: 59-69, 2017.
104. Garbayo E, Gavira JJ, de Yebenes MG, Pelacho B, Abizanda G, Lana H, Blanco-Prieto MJ and Prosper F: Catheter-based intramyocardial injection of FGF1 or NRG1-loaded MPs improves cardiac function in a preclinical model of ischemia-reperfusion. Sci Rep 6: 25932, 2016.
105. Díaz-Herráez P, Saludas L, Pascual-Gil S, Simón-Yarza T, Abizanda G, Prósper F, Garbayo E and Blanco-Prieto MJ: Transplantation of adipose-derived stem cells combined with neuregulin-microparticles promotes efficient cardiac repair in a rat myocardial infarction model. J Control Release 249: 23-31, 2017.
106. Bhagra SK, Pettit S and Parameshwar J: Cardiac transplantation: Indications, eligibility and current outcomes. Heart 105: 252-260, 2019.
107. Liu X, Gu X, Li Z, Li X, Li H, Chang J, Chen P, Jin J, Xi B, Chen D, *et al*: Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. J Am Coll Cardiol 48: 1438-1447, 2006.
108. Gao R, Zhang J, Cheng L, Wu X, Dong W, Yang X, Li T, Liu X, Xu Y, Li X and 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 55: 1907-1914, 2010.
109. Jabbour A, Gao L, Kwan J, Watson A, Sun L, Qiu MR, Liu X, Zhou MD, Graham RM, Hicks M and MacDonald PS: A recombinant human neuregulin-1 peptide improves preservation of the rodent heart after prolonged hypothermic storage. Transplantation 91: 961-967, 2011.
110. Harvey RP, Wystub-Lis K, del Monte-Nieto G, Graham RM and Tzahor E: Cardiac regeneration therapies-targeting neuregulin 1 signalling. Heart Lung Circ 25: 4-7, 2016.
111. Caillaud K, Boisseau N, Ennequin G, Chavanelle V, Etienne M, Li X, Denis P, Dardevet D, Lacampagne A and Sirvent P: Neuregulin 1 improves glucose tolerance in adult and old rats. Diabetes Metab 42: 96-104, 2016.
112. Pentassuglia L, Heim P, Lebboukh S, Morandi C, Xu L and Brink M: Neuregulin-1β promotes glucose uptake via PI3K/Akt in neonatal rat cardiomyocytes. Am J Physiol Endocrinol Metab 310: E782-E794, 2016.
113. Ennequin G, Boisseau N, Caillaud K, Chavanelle V, Gerbaix M, Metz L, Etienne M, Walrand S, Masgrau A, Guillet C, *et al*: Exercise training and return to a well-balanced diet activate the neuregulin 1/ErbB pathway in skeletal muscle of obese rats. J Physiol 593: 2665-2677, 2015.
114. Ennequin G, Boisseau N, Caillaud K, Chavanelle V, Etienne M, Li X, Montaurier C and Sirvent P: Neuregulin 1 affects leptin levels, food intake and weight gain in normal-weight, but not obese, db/db mice. Diabetes Metab 41: 168-172, 2015.