Gene and cytokine therapy for heart failure: molecular mechanisms in the improvement of cardiac function

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Nagai T, Komuro I. Gene and cytokine therapy for heart failure: molecular mechanisms in the improvement of cardiac function. Am J Physiol Heart Circ Physiol 303: H501–H512, 2012. First published July 9, 2012; doi:10.1152/ajpheart.00130.2012.—Despite significant advances in pharmacological and clinical treatment, heart failure (HF) remains a leading cause of morbidity and mortality worldwide. Many new therapeutic strategies, including cell transplantation, gene delivery, and cytokines or other small molecules, have been explored to treat HF. Recent advancement of our understanding of the molecules that regulate cardiac function uncover many of the therapeutic key molecules to treat HF. Furthermore, a theory of paracrine mechanism, which underlies the beneficial effects of cell therapy, leads us to search novel target molecules for genetic or pharmacological strategy. Gene therapy means delivery of genetic materials into cells to achieve therapeutic effects. Recently, gene transfer technology in the cardiovascular system has been improved and several therapeutic target genes have been started to examine in clinical research, and some of the promising results have been emerged. Among the various bioactive reagents, cytokines such as granulocyte colony-stimulating factor and erythropoietin have been well examined, and a number of clinical trials for acute myocardial infarction and chronic HF have been conducted. Although further research is needed in both preclinical and clinical areas in terms of molecular mechanisms, safety, and efficiency, both gene and cytokine therapy have a great possibility to open the new era of the treatment of HF.

myocardial infarction
lum \( Ca^{2+}\)-ATPase (SERCA), and phospholamban (PLN), play a pivotal role in \( Ca^{2+}\)-handling system (5).

Recently, our understanding of the molecules involved in \( \beta \)-adrenergic systems and \( Ca^{2+}\) handling has been advanced. Therefore, more appropriate HF therapies by using newly developed drugs or genes has been proposed. Currently, numerous small molecule chemicals that have ability to improve cardiac function or attenuate cardiac remodeling have been reported. These include G protein \( \beta \gamma \)-subunit (G\( \beta \gamma \)) inhibitors, cardiac myosin activators, neuregulin-1\( \beta \), thyroid hormone analog, and P2X4 purinergic receptor agonists and so on (112, 115). Although a careful elucidation of dose/regimen is required to prevent the potential risk of systemic adverse effects, some of the chemicals have advanced into clinical trials. A phase Ib study of elective allosteric activator of cardiac myosin omecamtiv mecarbil in patients with left ventricular (LV) systolic dysfunction hospitalized for acute HF is now enrolling patients (73). Phase II and phase III studies of a neuregulin-1\( \beta \) fragment and a phase I study of a full-length glycosylated recombinant human neuregulin-1\( \beta 3 \) are ongoing (109). Because a complete review of all HF drugs is beyond the scope of this review, the authors recommend recent excellent reviews of key cardiac signaling molecules that are potential drug targets to treat HF (115).

Gene therapy means the delivery of genetic materials into cells to achieve therapeutic effects. As mentioned above, the discovery of new molecular targets for HF therapy facilitate the clinical application of pharmaceutical manipulations; however, a number of these molecules cannot be intervened systemically, rather suitable for gene therapy. In addition, the heart is easily and selectively accessible by percutaneous catheter approaches so that the myocardium is ready for gene delivery system. Over the past decade, there has been a great advancement in cardiovascular gene transfer technology in terms of the transduction efficiency and safety of viral vectors, methods of vector delivery, and nonviral gene carriers (59, 110, 135). Nonviral vectors such as plasmid DNA, liposome-DNA, and polymer-DNA are less expensive and safe; however, viral vectors are superior to the nonviral vectors in terms of efficiency of myocardial transduction and long-term transgene expression. Currently, the most commonly used viral vector in clinical cardiovascular gene therapy is adenovirus (121). Adeno-associated virus (AAV) and retrovirus are also used in some clinical trials. There are the advantages and limitations in each vector. Recombinant human adenosinoviruses efficiently deliver and express their genomes in both dividing and nondividing cardiomyocytes. However, the immune response that results in the myocardial inflammation and elimination of infected cells is a large obstacle. AAV can induce stable and long-lasting gene expression compared with adenovirus because of its minimal immunogenicity and particular serotypes, which has excellent tropism for the heart. The major disadvantage of AAV is their limited genome packing capacity. In addition, like adenovirus, the presence of neutralizing antibodies against some serotypes limits the efficiency of gene therapy with AAV. Retrovirus has the advantage in high-transduction efficiency and stable transduction into dividing cells; however, vector genome integration into the host genome raise a concern for the risk of oncogenic transformation. Lentivirus resembles retrovirus in its ability to stably integrate into the target cell genome. In contrast to retrovirus, lentivirus can transduce larger transgenes into nondividing cells. It is possible to minimize the risk of oncogenic transformation by modifying the vector design (75). Because ischemic heart disease is the most common cause of HF, the initial aim of gene therapy was targeted to restore the coronary vasculature. Genetic transfer of angiogenic growth factors or cytokines has been evaluated on animal models, and several controlled clinical trials for ischemic heart disease have been published including VEGF-A165, VEGF-121, VEGF-C, and FGF-4 (66). Along with elucidating multiple steps at which \( \beta \)-AR signaling systems and excitation-contraction coupling are dysregulated, various targets for gene therapy such as G protein-coupled receptor kinase 2 (GRK2), adenylyl cyclase type 6 (AC6), SERCA2a, PLN, inhibitor protein (IN) 1 and 2, and S100A1 have been emerged. Preclinical data suggest that these molecules are important to restore the normal cardiac function. In the first part of this review, we will focus on the recent advancement of the gene therapy targeting on the above seven molecules.

Various growth factors and cytokines, such as granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), and insulin-like growth factor-1 have been reported to prevent cardiomyocytes from apoptosis as well as promote angiogenesis (12, 40, 89). Recently, particular interest has been focused on mesenchymal stem cells (MSCs) from bone marrow or fat tissue as potential cell therapy candidates (130). It has been reported that MSCs secrete a variety kind of cytokines, which inhibit apoptosis, inflammatory cascade, and degradation of extracellular matrix, leading to cardiac repair (97). Preclinical studies have shown the improvement in cardiac function after administration of MSC-conditioned medium (33). Secretome analysis of MSCs under stimulated conditions, such as hypoxia or pharmacological and genetic modulation, is expected to unveil novel paracrine factors (97). These findings prompt the clinical trials of cytokine therapy for treating cardiovascular diseases as cell-free therapy alternate to using MSCs. In contrast to newly developed pharmacological chemicals, cytokines are a component of intrinsic factors, and several cytokines have already been used for human hematopoietic or autoimmune diseases, suggesting less concern about safety for clinical application. Therefore, a proper use of cytokines may be a promising therapeutic strategy against HF. In the second part of this review, we focus on the results of recent clinical trials of cytokine therapy, especially of G-CSF and EPO, and introduce the current understanding of their beneficial mechanisms.

**Gene Therapy Targeted on \( \beta \)-Adrenergic Signaling**

\( \beta \)-ARs are seven transmembrane-spanning G protein-coupled receptors. \( \beta \)-ARs are comprised of three subtypes: \( \beta 1 \), \( \beta 2 \), and \( \beta 3 \)-ARs. In the heart \( \beta 1 \)-ARs are the predominant subtype. Activation of \( \beta \)-ARs in response to sympathetic neurotransmitters such as epinephrine and norepinephrine results in the dissociation of the stimulatory G protein \( \alpha \)-subunit (Gos) from G\( \beta \gamma \) (103). Gas stimulates AC to produce adenosine 3',5'-cyclic monophosphate (cAMP) from ATP, leading to the activation of cAMP-dependent PKA, locally bound by an A kinase-anchoring protein. PKA regulates different intracellular, sarcolemmal, and myofibrillar substrates, resulting in the increasing of \( Ca^{2+}\) influx and efflux and actin-myosin interaction. Therefore, \( \beta 1 \)-ARs play a critical role in the regu-
GRK2 phosphorylates GRK2 translocation and binds to its carboxyl-terminal domain. Clarified as a selective reduction of \( H9252 \) system (11). The molecular mechanism of activating transcription factor-3. \( = - \text{cyclic } \)

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**G protein-coupled receptor kinase 2.** In the failing heart, one of the biological defects is a significant alteration of \( \beta \)-AR system. The molecular mechanism of \( \beta \)-AR dysfunction is clarified as a selective reduction of \( H9252 \) system (11). The molecular mechanism of activating transcription factor-3.

Figure 1 shows a schema of \( \beta \)-AR signaling pathway and current target of gene therapy in \( \beta \)-AR signaling pathway.

**Figure 1.** Cardiomyocyte \( \beta \)-adrenergic receptor (\( \beta \)-AR) signaling during heart failure (A) and after treatment with carboxy-terminus of \( \beta \)-AR kinase (\( \beta \)-ARKct) and adenylyl cyclase type 6 (AC6) gene therapy (B). A: G protein-coupled receptor kinase 2 (GRK2) phosphorylates \( \beta \)-AR and facilitates the recruitment of \( \beta \)-arrestin, resulting in \( \beta \)-AR downregulation and impairment of further G protein activation. B: \( \beta \)-ARKct inhibits the recruitment of GRK2, \( \beta \)-ARKct inhibits the recruitment of GRK2, \( \beta \)-ARKct inhibits the recruitment of GRK2, \( \beta \)-ARKct inhibits the recruitment of GRK2, dissolves the interaction of GRK2 with \( \beta \)-ARs, and promotes normalization of cAMP signaling. AC6 augments Ca\(^{2+}\) handling via protein kinase A (PKA)-dependent and -independent pathway. ATP, adenosine 5’-triphosphate; cAMP, adenosine 3’,5’-cyclic monophosphate; CA, catecholamine; PI3, phosphatidylinositol 3; ATF3, activating transcription factor-3.

**ABSTRACT**

In the failing heart, one of the biological defects is a significant alteration of \( \beta \)-AR system. The molecular mechanism of \( \beta \)-AR dysfunction is clarified as a selective reduction of \( H9252 \) system (11). The molecular mechanism of activating transcription factor-3. Detailed pathway.

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and cAMP production were enhanced in response to β₁-AR agonist, and levels of B-type natriuretic peptide were reduced (65). Currently, a phase-I/II study of human adenovirus-5 encoding human AC6 gene transfer in patients with HF began enrollment (ClinicalTrials.gov, NCT00787059). The study was a randomized, double-blinded, placebo-controlled study. The vector will be delivered by intracoronary injection with dose escalation. Recently, cAMP-independent effects of AC6 on cardiomyocytes have been reported. These included an increase in activating transcription factor-3 expression, leading to reduced PLN transcription and an increase in phosphatidylinositol 3-kinase (PI3K)/Akt activation, resulting in increased PLN phosphorylation and expression of Bcl-2 protein (32). Although the contribution of these mechanisms is uncertain in the failing human heart, the multifunctional roles strengthen the value of AC6 as the target of gene therapy.

**Gene Therapy Targeted on Excitation-Contraction Coupling**

The Ca²⁺ handling during contraction and relaxation is a prominent feature of cardiomyocytes (5). In brief, membrane depolarization triggers Ca²⁺ influx through L-type Ca²⁺ channels, followed by a Ca²⁺-induced Ca²⁺ release through the RyR of SR. The elevated cytosolic Ca²⁺ binds to troponin C and activates the contraction unit. During the systolic period, SR Ca²⁺ content is being depleted, followed by an inactivation of RyRs, and cardiomyocytes then turn from a systolic to diastolic mode. During the diastolic period, the cardiac SERCA2a and sarcolummal Na⁺/Ca²⁺ exchanger are the major mechanisms in Ca²⁺ extrusion. Ca²⁺ uptake through the SERCA pump is negatively regulated by PLN. The amplitude and frequency of the Ca²⁺ transient are regulated by the phosphorylation status of Ca²⁺ cycling regulators, which depends on the balance between the activity of kinase and phosphatase. PKA and Ca²⁺/calmodulin-dependent protein kinase II (CaMK II) are essential kinases in cardiomyocytes. PKA is activated by β-AR signal, which is mediated by AC and cAMP formation. PKA phosphorylates L-type Ca²⁺ channels and RyR, which enhances Ca²⁺ influx and promotes SR Ca²⁺ release, respectively. PKA also phosphorylates and inactivates PLN, resulting in the augmentation of SERCA pump activity. Proteins phosphorylated by PKA or CaMK II are actively dephosphorylated by phosphatases such as protein phosphatase (PP) 1 and 2A. Cytosolic PP1 activity is regulated by IN-1 and IN-2, which are activated by PKA phosphorylation and works as an amplifier of β-AR signaling. Figure 2 shows a schema of Ca²⁺ signaling during excitation-contraction coupling and current targets of gene therapy in Ca²⁺ cycling regulators.

**SERCA2a.** Since Hasenfuss et al. (43) reported that a loss of activity of SERCA2a and subsequent decrease in SR Ca²⁺ uptake are a feature of the failing human heart, the SERCA2a becomes one of the most studied Ca²⁺ handling proteins.

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**Fig. 2.** Target molecules for gene therapy in Ca²⁺ cycling within cardiomyocytes. The amplitude and velocity of the Ca²⁺ transient is regulated by the balance between phosphorylation (P) and dephosphorylation of Ca²⁺ cycling regulators in cardiomyocytes. PKA and Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) are major kinases, positively regulating Ca²⁺ cycling. Protein phosphatases (PP) 1 and 2A are major phosphatases, negatively regulating Ca²⁺ cycling. The expected candidate molecules for gene therapy, including sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA2A), phospholamban (PLN), inhibitor (IN)-1 and -2, and S100A1 are illustrated. Red arrows indicate the direction of Ca²⁺ movement. Dotted red arrows indicate the decline of Ca²⁺ movement. LTCC, voltage-operated L-type Ca²⁺ channels; NCX, sarcolemmal Na⁺/Ca²⁺ exchanger; RyR, ryanodine receptor.
targeting for gene therapy. Overexpression of SERCA2a gene in human failing cardiomyocytes induced a faster contraction and enhanced relaxation velocity (18). The therapeutic potential of SERCA2a gene transfer to failing heart has been evaluated in small animal model of HF. Intracoronary adenovirus-encoding SERCA2a delivery to rats in HF resulting from pressure overload showed improved systolic and diastolic function (19, 107). A direct injection of adenovirus-encoding SERCA2a into myocardium prevented decrease of dP/dt max and dP/dt min, restored wall thickness, and reduced infarct size in rat ischemic-reperfusion model (20). The studies with preclinical large animal models of HF have also proved the therapeutic potential of SERCA2a gene delivery. In a pig model of volume-overload HF due to mitral regurgitation, intracoronary delivery of recombinant AAV1 carrying SERCA2a improved adjusted dP/dt max and LV function (58). In sheep, a HF model induced by MI with a mitral regurgitation model, intracoronary delivery of AAV6 carrying SERCA2a improved dP/dt max and LV remodeling (4). There were two reports, which contradict the above findings. In transgenic rats with cardiac SERCA2a overexpression, there was no beneficial effect on LV function after MI and even an increased mortality due to ventricular arrhythmia (14). Gene transfer of SERCA2a to isolated cardiomyocytes from a canine LV pressure-overload diastolic HF model improved diastolic function, however, but abrogated β-adrenergic responsiveness and inotropic support (47). Although it has been subsequently reported that proarrhythmic effect is not observed and rather arrhythmias were attenuated in porcine model of ischemia-reperfusion model after SERCA2a gene therapy (95), caution must be taken when gene therapy is applied to HF with normal ejection fraction and MI.

Recently, the first clinical trial of SERCA2a myocardial gene therapy in patients with HF was launched. Calcium Uprgulation by Percutaneous Administration of Gene Therapy in Cardiac Disease Trial (CUPID) is designed to evaluate the safety profile and the biological effects of gene transfer of the SERCA2a cDNA by intracorony delivering of AAV1/SERCA2a in patients with advanced HF. This study compares three doses of AAV1/SERCA2a with placebo in patients with advanced HF (New York Heart Administration class III and IV), a LV ejection fraction ≤ 35% of both ischemic and nonischemic dilated cardiomyopathy. In a phase I/II trial, the safety and feasibility of AAV1/SERCA2a administration have been demonstrated (54). Recently, Jessup et al. (55) have reported the results of a phase II trial, in which 39 patients were enrolled. At 6 mo, AAV1/SERCA2a high-dose group showed clinically significant improvements in patients’ symptoms and functional status, as well as a significant reduction of cardiovascular events and hospitalization times. This was supported by a reduction in NH2-terminal prohormone brain natriuretic peptide and improvement of ventricular function/remodeling (55). In this phase II trial, 509 patients were prescreened and 63 patients with undetectable neutralizing antibodies against AAV were considered as candidates because patients who had pre-existing anti-AAV neutralizing antibodies did not respond to AAV1/SERCA2a in earlier phase I/II trial. Although a relatively high incidence of the presence of neutralizing antibodies limits the efficacy of gene therapy, SERCA2a gene therapy seems to be promising and the larger trial is awaited.

Phospholamban. In consideration of the good outcomes from a CUPID trial, PLN, a key negative regulator of SERCA2a, also looks like a candidate for therapeutic target of gene therapy for HF. Indeed, transgenic mice overexpressing a mutant PLN superinhibitor of SERCA2a in cardiomyocytes suffered cardiomyopathy (36), whereas PLN knockout mice showed enhanced systolic and diastolic function (71). However, the results of studies, which were designed to rescue HF by ablation of PLN, are variable. Muscle LIM protein-deficient and calsequestrin-overexpressing mice showed the improvement of cardiac function and Ca2+ transient when crossed bred with PLN knockout mice (77, 108). On the contrary, cardiac ablation of PLN did not rescue the hypertrophic phenotype of β2-AR overexpressing and mutant myosin-binding protein C mice and did not improve the cardiac dysfunction of mice with cardiac-specific overexpression of tumor necrosis factor-α, although cellular contractility showed significant improvement (29, 53, 116). Adenoviral gene transfer of antisense PLN prevented contractile dysfunction in Ca2+ overload-induced LV dysfunction (123). Gene transfer of recombinant AAV encoding phosphomimetic mutant (S16E) form of PLN enhanced myocardial SR Ca2+ uptake and suppressed progressive impairment of LV systolic function in B1014.6 cardiomyopathic hamsters (49). Intracorony delivery of adenovirus expressing PLN-S16E demonstrated improved cardiac function in sheep pacing-induced HF (60). However, cardiac gene transfer of AAV6 expressing short hairpin RNA against PLN resulted in depressed cardiac function along with reduction of PLN protein (7). These findings suggest that endogenous PLN may play a protective role in the heart in some context. Of note, there are several reports of familial human cardiomyopathies, which are caused by mutations and deletions in the PLN gene or its promoter and where the outcome is a loss of PLN inhibition on SERCA2a (37, 109a). Therefore, before human gene therapy targeting on PLN lowering is launched, more studies on PLN might be warranted.

IN-1 and IN-2. Expression levels and activity of IN-1 and IN-2 are decreased in HF, which is associated with the increase in global and SR-associated PP1 activity, leading to depressed SR Ca2+ pump activity (25, 34, 39). Overexpression of IN-1 in cardiomyocytes augmented Ca2+ cycling and cell contraction/relaxation in response to β-AR signaling (24). Adenoviral gene delivery of constitutively active IN-1 has been shown to augment cardiac contractility, attenuate hypertrophy, and prevent HF (90). In addition, cardiac-specific overexpression of IN-2 in mouse and gene delivery of IN-2 in cardiomyopathic hamster increased cardiac contractility by augmenting Ca2+ cycling (61, 134). However, Wittkopper et al. (132) have recently reported that conditional cardiomyocyte-restricted expression of constitutively active mutant form of IN-1 increases contractile function in the cost of lethal arrhythmia and exaggeration of cardiomyopathy after adrenergic stress and with aging. The authors (132) confirmed similar phenotypes in another line of transgenic mice, which expressed an active form of IN-1 resistant to PKC-α phosphorylation. The authors attributed their results to RyR2 phosphorylation in both of Ser2809 (PKA site) and Ser2815 (CaMKII site) in their transgenic mice. RyR2 phosphorylation in Ser2809 has been previously reported and related to beneficial effects; however, phosphorylation in the CAMKII site has not been examined so far. Phosphorylation of RyR2 at Ser2815 may increase Ca2+ fre-
and reverses cardiac dysfunction and LV remodeling along noninfarcted myocardium in pig chronic MI model prevents have reported that delivery of AAV9-S100A1 to the LV farcted failing rat heart (82, 93). Recently, Pleger et al. (94) reported that G-CSF stimulates bone marrow cells to mobilize glycoprotein cytokine that stimulates the proliferation and function and attenuate LV remodeling via angiogenesis, anti-

Although our understanding of how S100A1 organizes cardio-
metering proteins, 1) SR Ca^{2+}- handling proteins, 2) cardiac titin, and 3) mitochondrial proteins (104). S100A1 enhances diastolic Ca^{2+} uptake from SERCA2a, diminishes diastolic Ca^{2+} leakage, and increases systolic Ca^{2+} release through RyR interaction with the RyR and the SERCA2/PLN complex. S100A1 regulates titin-actin interaction results in the reduction of titin-mediated vicious break and improvement of myofilament sliding. Additionally, S100A1 enhances ATP production through interaction with inner mitochondrial membrane and matrix molecules. Although our understanding of how S100A1 organizes cardiomyocyte Ca^{2+} dynamics, structure and metabolism is still incomplete and further studies are warranted, S100A1 may be a promising therapeutic target on HF.

Cytokine Therapy

Many of the growth factors, cytokines, and receptors have been reported to be potent as a therapeutic regimen for cardiac repair following MI. Most of the factors improve cardiac function and attenuate LV remodeling via angiogenesis, anti-apoptotic effects, and stem cell homing. Among these factors, G-CSF and Epo are extensively studied, and several clinical trials are achieved.

Granulocyte colony-stimulating factor. G-CSF is a 25-kDa glycoprotein cytokine that stimulates the proliferation and survival of granulocytes lineage-derived cells. Orlic et al. (86) reported that G-CSF stimulates bone marrow cells to mobilize into the infarcted area and to differentiate into cardiomyocytes in mice. However, there is still a controversy about G-CSF-mediated cardiac regeneration by bone marrow stem cells (22, 78). The mechanisms other than cardiac regeneration have emerged from the findings that G-CSF receptor is expressed on cardiomyocytes and endothelial cells and that its downstream Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway is essential for the effect of G-CSF in the heart (29). It has been reported that G-CSF reduces the number of apoptotic cells in the ischemic border zone and increases Akt activities in the myocardium in mice, rats, and swines in an acute phase of MI (52, 85, 117). G-CSF also increased the production of nitric oxide (NO) through Akt/endothelial NO synthase pathway and enhanced the proliferation and migration of endothelial cells (88, 124). Neutrophils and macrophages play important roles in accelerating the infarct healing process. G-CSF-mediated infiltration of inflammatory cells is the first step to regulate cardiac healing. Fujita et al. (30) have reported that G-CSF mobilizes monocytes/macrophage, which differentiate into myofibroblasts and enhance cardiac repair. G-CSF treatment improved early postinfarct ventricular expansion through enhancement of expression of transforming growth factor-β, and promotion of reparative collagen synthesis in the infarcted heart (92). Although there was a conflicting report showing that G-CSF reduces ratio of metalloproteinases to their tissue inhibitors and aggravates excessive fibrosis deposition in infarcted areas of the myocardium (15), the more sophisticated protocol of dose and duration of G-CSF treatment may ensure the beneficial effect on the infarct healing process. Recently, several reports suggest that G-CSF exerts pleiotropic effects on cardiac tissue such as improvement of impaired mitochondrial electron transport and oxygen consumption (46). Cardioprotective effects of G-CSF have been reported in chronic myocardial ischemia and HF through angiogenesis (42, 17), reduction of apoptosis (42), prevention of the formation of excessive granulation tissue (17), and reduced expression of the angiotensin II type-1 receptor and tumor necrosis factor-α (69). These results highlight that the effects of G-CSF treatment might also occur in the chronic phase through similar or distinct mechanism in acute phase. Figure 3 showed a diagram of cellular and molecular interaction in cardiac repair after G-CSF therapy after MI.

Many clinical studies using G-CSF for patients of acute MI have been reported. Table 1 shows a summary of clinical studies with G-CSF in infarcted patients. Since the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor Trial (FIRSTLINE-AMI) first reported that patients receiving G-CSF had increased mobilization of CD34+ mononuclear cells, had improved ejection fraction, and had prevented LV remodeling without safety concerns (51), several randomized, controlled trials have been conducted; however, the results of these trials are discordant, and recent published reports of the meta-analysis of the randomized controlled trials are still inconclusive. Kang et al. (56) have reported that G-CSF treatment improves LV ejection fraction in acute MI from 14 randomized controlled trials (56). The result of the meta-analysis by Zohlnhöfer et al. (137) has shown that G-CSF treatment does not enhance the improvement of ejection fraction from 10 randomized controlled trials. Although clinical trials have shown equivocal results, stem cell
mobilization with G-CSF is still promising because of its feasibility and general applicability. In addition to an elucidation of the underlying mechanisms, additional careful and well-designed protocols in terms of dose of G-CSF, timing of the treatment, and profiles of patients are still necessary to examine because it has been reported that the degree of improvement in LV ejection fraction is inversely associated with the time to G-CSF administration in patients after reperfusion and LV ejection fraction at baseline (1, 120).

**Erythropoietin.** Erythropoietin (Epo) exerts its hematopoietic effects by stimulating the proliferation of early erythroid precursors and the differentiation of later precursors of the erythroid lineage (64). Independent of erythropoiesis, recently EPO was shown to protect the heart from ischemic injury (77, 118). EPO receptor was expressed in both ventricular myocytes and endothelial cells (21). The interaction between EPO and its receptors leads to dimerization of the receptors and activation of signaling pathways, including STAT5, PI3K/Akt, and mitogen-activated protein kinase (MAPK). Erythropoietin pre-activates endothelial NO synthase, serving to improve mitochondrial function (48). Besides protective and angiogenic effects of EPO, it has been recently reported that EPO activates Janus-activated tyrosine kinase 2 (JAK2) and its downstream antiapoptotic and angiogenic signaling through STAT, PI3K/Akt, and MAPK signaling. G-CSF promotes early myocardial healing through enhancement of transforming growth factor-β (TGF-β1) and prevents later excessive fibrosis through downregulation of angiotensin II type-1 receptor (AT1R) and TNF-α. G-CSF improves impaired mitochondrial (Mt) function. EPO induce secretion of sonic hedgehog (Shh) from cardiomyocyte and cardiomyocyte itself secretes EPO. These factors also contribute to vascular formation. Ang-1, angioptin1; CPC, cardiac progenitor cell; eNOS, endothelial nitric oxide (NO) synthase; HSC, hematopoietic stem cell; Mo, macrophage; MAPK, mitogen-activated protein kinase; Mo, monocyte; PKC, protein kinase C; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

**Table 1. Overview of randomized controlled trials with G-CSF in infarcted patients**

<table>
<thead>
<tr>
<th>Trial</th>
<th>N</th>
<th>Age, yr</th>
<th>Dose, µg/kg (days)</th>
<th>Cardiac function measurement</th>
<th>Mean follow-up duration, mo</th>
<th>Outcomes</th>
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<tr>
<td>Ince et al. (51)</td>
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<td>50</td>
<td>10 (6)</td>
<td>ECHO</td>
<td>4</td>
<td>LVEF↑, MACE→</td>
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<tr>
<td>Zohlnhöfer et al. (136)</td>
<td>114</td>
<td>59</td>
<td>10 (5)</td>
<td>MRI</td>
<td>4</td>
<td>LVEF→, MACE→</td>
</tr>
<tr>
<td>Valgimigli et al. (127)</td>
<td>20</td>
<td>60</td>
<td>5 (4)</td>
<td>SPECT</td>
<td>6</td>
<td>LVEF→, MACE→</td>
</tr>
<tr>
<td>Ripa et al. (101)</td>
<td>78</td>
<td>56</td>
<td>10 (6)</td>
<td>MRI</td>
<td>6</td>
<td>LVEF→, MACE→</td>
</tr>
<tr>
<td>Engelmann et al. (27)</td>
<td>44</td>
<td>58</td>
<td>10 (5)</td>
<td>MRI</td>
<td>3</td>
<td>LVEF→, MACE→</td>
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<tr>
<td>Takano et al. (119)</td>
<td>40</td>
<td>62</td>
<td>2.5 (5)</td>
<td>SPECT</td>
<td>6</td>
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<tr>
<td>Leone et al. (67)</td>
<td>41</td>
<td>55</td>
<td>10 (5)</td>
<td>ECHO</td>
<td>6</td>
<td>LVEF↑, MACE→</td>
</tr>
</tbody>
</table>

* N represents the total number of patients. Age is shown as mean. G-CSF, granulocyte colony-stimulating factor; ECHO, echocardiography; LVEF, left ventricular ejection fraction; MACE, major adverse cardiac events; MRI, magnetic resonance imaging; SPECT, single-photon emission computed tomography.
diagram of cellular and molecular interaction in cardiac repair after EPO therapy after MI.

A single bolus of EPO in patients with acute MI showed that EPO was safe and well tolerated (70). EPO stimulated endothelial progenitor cell mobilization; however, LV ejection fraction was similar between the EPO-treated and control groups in this pilot study. Subsequently, A Prospective, Randomized, Clinical Study to Examine the Effects of a Single Bolus Erythropoietin on Left Ventricular Function in Patients with an Acute Myocardial Infarction (HEBE-III) was initiated to assess the effects of EPO in a larger number of acute MI patients. The results showed that a single dose of EPO after successful percutaneous coronary intervention did not improve LV ejection fraction, but the treatment with EPO related less major adverse cardiovascular events and a favorable clinical safety profile (128). No improvement on cardiac function or reduction in infarct size was observed in A Randomized, Double-Blind, Placebo-Controlled Trial of Erythropoietin in Patients with ST-Segment Elevation Myocardial Infarction Undergoing Percutaneous Coronary Intervention (REVIVAL-3), which was another prospective, randomized, double-blind, placebo-controlled trial of EPO in patients with acute MI (87).

Currently, the Reduction of Infarct Expansion and Ventricular Remodeling with Erythropoietin after Large Myocardial Infarction Trial (REVEAL) and Exogenous erythropoietin in Acute Myocardial Infarction: New Outlook and Dose Association Study (EPAMINONDAS) trials are ongoing (2, 76). Table 2 shows a summary of clinical studies with EPO in infarcted patients. Despite the lack of beneficial effects of EPO on cardiac function in patients of MI, treatment with erythropoiesis-stimulating agents for chronic HF with anemia showed the improvement of LV ejection fraction along with better exercise tolerance and reduction of symptoms (63). Since it has been reported that there is an ideal dose of EPO for MI (134), the results of further clinical trials may provide us a new insight of the safety and efficacy of EPO in cardiovascular disease.

Summary

HF is a major clinical cause of morbidity and mortality, and the need for better treatments has been recognized for decades. Many new approaches to HF therapy including cells, drugs, and genes have been explored, and it is important to note that these therapies are complementary. Gene therapy means the delivery of genetic materials into cells to achieve therapeutic effects. AAV own attractive characters such as the defective replication, lack of human pathology, and prolonged transgene expression in clinical studies. Because β-adrenergic signaling and excitation-contraction coupling are crucial for cardiac contraction, gene therapy targeting for GRK2, AC6, SERCA2a, PLN, IN-1, IN-2, and S100A1 have shown promising preclinical data. Clinical trials of AC6 and SERCA2a have launched. A growing amount of evidence indicates that G-CSF and EPO promote cardiac repair after MI through mobilization of the stem or progenitor cells from bone marrow and also through direct pleiotropic cardiovascular effects such as antiapoptosis, angiogenesis, regulation of fibrosis, and improved mitochondrial function. The preclinical studies have been actively investigated with promising data, which show that G-CSF and EPO improve cardiac function in various kinds of models of HF.

Future Directions

A recent advance in our understanding of the molecular mechanisms associated with HF clarifies several key molecules, which are promising targets for gene therapy. Gene therapy is considered as a viable adjunctive treatment to mechanical and pharmacological therapies for HF; however, further development of advanced vectors based on elaborated preclinical experiments by using large animals are still needed. The more favorable characters of next generation vectors include the regulated expression pattern by which we can manipulate the on/off system or environment-dependent expression such as tetracycline regulatory system and hypoxia-inducible factor-1α-dependent gene expression. Because of the complexity of the pathophysiology of HF, the more target molecules that are amenable to genetic manipulations have to be considered. These include key molecules that govern the homing and differentiation of stem cells, cell death/survival, and myocyte cell cycle. Further studies along with the development of more selective and noninvasive vector delivery systems will undoubtedly lead to safer and more effective gene therapy for HF.

Although most of the meta-analyses suggest that the administration of G-CSF or EPO does not improve the beneficial effects of standard HF therapy, safety of the usage of G-CSF and EPO has been demonstrated. In addition, some methodological issues, such as patient age, time of onset of symptoms to percutaneous coronary intervention, and time to administration of cytokines, need to be carefully addressed. To clarify the efficacy of G-CSF and EPO treatment, the establishment of better protocols in terms of the appropriate indications, doses, therapeutic window, as well as more mechanistic insight of molecular pathways under G-CSF and EPO are needed.

Table 2. Overview of randomized controlled trials with EPO in infarcted patients

<table>
<thead>
<tr>
<th>Trial</th>
<th>N</th>
<th>Age</th>
<th>Dose</th>
<th>Cardiac function measurement</th>
<th>Mean follow-up duration, mo</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipson et al. (70)</td>
<td>50</td>
<td>50</td>
<td>300 μg (≈60,000 IU) darbepoetin-α single bolus IV</td>
<td>Radionuclide ventriculography</td>
<td>4</td>
<td>LVEF→, MACE→</td>
</tr>
<tr>
<td>Voors et al. (128)</td>
<td>529</td>
<td>61</td>
<td>60,000 IU epoietin-α single bolus IV</td>
<td>Radionuclide ventriculography</td>
<td>1.5</td>
<td>LVEF→, MACE→</td>
</tr>
<tr>
<td>Ott et al. (87)</td>
<td>138</td>
<td>61</td>
<td>33,300 IU epoietin-β single bolus IV</td>
<td>Angiography</td>
<td>6</td>
<td>LVEF→, MACE→</td>
</tr>
<tr>
<td>Melloni et al. (76)</td>
<td>210</td>
<td></td>
<td>15, 30, or 60 × 10^7 IU epoietin-α single bolus IV</td>
<td>MRI</td>
<td>3</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Andreotti et al. (2)</td>
<td>102</td>
<td></td>
<td>100 or 200 IU·kg⁻¹·day⁻¹ epoietin-α triple 30 min IV</td>
<td>MRI</td>
<td>12</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

N represents the total number of patients. Age is shown as mean. EPO, erythropoietin; IU, international units; IV, intravenous.
DISCLOSURES

No conflicts of interest or otherwise are declared by the author(s).

AUTHOR CONTRIBUTIONS

T.N. and I.K. conception and design of research; T.N. drafted manuscript; I.K. edited and revised manuscript; I.K. approved final version of manuscript.

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Review


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