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

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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

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Introduction

Despite significant advances in pharmacological and clinical treatment, heart failure (HF) remains a leading cause of morbidity and mortality worldwide. Numerical evidence suggests that HF is a progressive disorder of which pathogenesis is related to many factors such as ischemia, mechanical stress, inflammation, metabolic disorders, and genetic disorders (74). Many new therapeutic strategies, including cell transplantation, gene delivery, and cytokines or other small molecules, have been explored to treat the various pathophysiological status of HF. Functional improvement after cell transplantation in the failing heart after ischemia has been demonstrated in preclinical and clinical studies for different subsets of bone marrow-derived cells and other adult progenitor cells, as well as cardiac stem cells, embryonic or induced pluripotent stem cells, are considered as cellular sources for generating cardiomyocytes (68, 133). Thus cell therapy is a promising approach for the treatment of HF. One of the most recent hypotheses is that the transplanted cells exert their beneficial effect via a paracrine mechanism in which molecules from transplanted cells may promote angiogenesis, cell survival, stem cell homing, myocardial contraction, and cardiomyogenesis (33). A quantitative proteomic approach and secretome analysis of transplanted cells may enable us to uncover the target molecules for pharmacological or genetic strategy. Since the particular drug or gene therapy may enhance the effect of cell therapy on cardiac regeneration, cell and pharmacological or gene therapy are complementary.

HF is characterized by impaired contraction and relaxation of the affected ventricles. Recently, great attention has been paid to two molecular pathophysiological abnormalities in failing myocytes: downregulation and desensitization of β-adrenergic receptors (β-ARs) (10, 26) and alterations of intracellular Ca2+ handling (6, 44). Although treatment with β-blockers improved the prognosis of HF, other pharmacological therapy intended to reverse or bypass β-adrenergic desensitization, such as phosphodiesterase inhibitors, catecholamines, or other positive inotropic agents, showed symptomatic improvement but increased mortality in patients (28). Similarly, transgenic overexpression of β-ARs, G proteins, or protein kinase A (PKA) caused short-term improvements in cardiac function but long-term cardiac dysfunction (23, 26). Ca2+ transients in failing cardiomyocytes are characterized by a lower amplitude and slower decline compared with those of normal cardiomyocytes (5, 50). It has been reported that sarcoplasmic reticulum (SR) and its membrane-anchored proteins, ryanodine receptors (RyRs), sarco(endo)plasmic reticu-
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 β-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 βγ-subunit (Gβγ) inhibitors, cardiac myosin activators, neuregulin-1β, 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 IIb 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-1β fragment and a phase I study of a full-length glycosylated recombinant human neuregulin-1β 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 pharmacological 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 adenoviruses 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 its 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 β-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 various 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 β-Adrenergic Signaling**

β-ARs are seven transmembrane-spanning G protein-coupled receptors. β-ARs are comprised of three subtypes: β1-, β2-, and β3-ARs. In the heart β1-ARs are the predominant subtype. Activation of β-ARs in response to sympathetic neurotransmitters such as epinephrine and norepinephrine result in the dissociation of the stimulatory G protein α-subunit (Gαs) from Gβγ. (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, β1-ARs play a critical role in the regu-
ylation of cardiac chronotropy, inotropy, and lusitropy. Figure 1 shows a schema of β₁-AR signaling pathway and current target of gene therapy in β₁-AR signaling pathway.

G protein-coupled receptor kinase 2. In the failing heart, one of the biological defects is a significant alteration of β-AR system (11). The molecular mechanism of β-AR dysfunction is clarified as a selective reduction of β₁-AR density at the plasma membrane (downregulation) and by an uncoupling of β₁-ARs from G proteins (desensitization) (9, 72). GRK2 are serine/threonine kinases, which consist of a central catalytic domain (~270 amino acids) flanked by an amino-terminal domain (~185 amino acids) and a variable-length carboxyl-terminal domain (~105–230 amino acids). In the heart, GRK2 is a predominant form. When catecholamine occupies β₁-AR and Gα dissociates from Gβγ, the remaining Gβγ facilitates GRK2 translocation and binds to its carboxyl-terminal domain. GRK2 phosphorylates β₁-AR and enhances the affinity for binding to β-arrestins, which prevents further G protein activation and induces the endocytic process of β₁-AR (16, 91). It has been reported that the expression and activity of GRK2 are significantly elevated in human HF (126). Therefore, the potential effects of GRK2 inhibition on in vivo model were extensively studied.

Koch et al. (62) developed carboxy-terminus of β-AR kinase (β-ARKct) peptide, which competes with endogenous GRK2 for binding to Gβγ and works as a GRK2 inhibitor. Mice overexpressing the β-ARKct peptide displayed enhanced cardiac contractility in vivo with or without isoproterenol (62). Subsequent studies showed that overexpression of β-ARKct improves cardiac function in a murine model of HF, such as muscle LIM protein-deficient and calsequestrin-overexpressing mice (41, 102). The beneficial effects of β-ARKct in HF were also observed in species other than the mouse. Intracoronary delivery of adeno virus encoding β-ARKct into rabbit HF model after myocardial infarction (MI) revealed the improvement of systolic function and the increase in β₁-AR density and AC activity (111). Rengo et al. (100) have reported that long-term suppression of GRK2 by delivery of recombinant AAV serotype 6 (AAV6) encoding β-ARKct improves cardiac contractility and reverses LV remodeling in the rat HF model after MI. Furthermore, adenovirus-mediated β-ARKct gene transfer in failing human ventricular myocytes increased the contraction and relaxation velocities as well as AC activity in response to β-agonist (131). Recently, Katz et al. (57) have reported successful delivery of self-complementary AAV6 encoding β-ARKct into the normal sheep heart by using molecular cardiac surgery with recirculating delivery system. The heart treated with AAV6 encoding β-ARKct significantly increased the maximum values of the first derivative of LV pressure (dP/dt max) compared with control and moderate increase in cAMP and β₁-AR density (57). Raake et al. (96) delivered AAV6 encoding β-ARKct by retrograde injection into the anterior interventricular vein of porcine model of HF after MI. Treatment with β-ARKct significantly achieved improved LV hemodynamics and contractile function in HF pigs (96). Although further studies with preclinical large animal models of HF are needed, β-ARKct gene therapy in human HF seems to be promising.

Adenylyl cyclase type 6. AC6 regulates the conversion of ATP to cAMP, leading to the activation of PKA and initiating a variety of intracellular signaling cascades that influence heart function. The general structure of AC consists of two transmembrane regions and two cytosolic loops. Cytosolic loops comprise the catalytic core, a primary site for the regulation of AC activity. A dominant isoform expressed in mammalian cardiac myocytes is AC6 and AC5 (39, 92). Gao et al. (31) have reported that transgenic mice with cardiac-directed expression of AC6 show normal cAMP production, cardiac function, myocardial β-AR number, and G protein content, expect for an increase in GRK2 content. Under the stimulation through the β-AR, cardiac function and cAMP production were increased (31). It is noteworthy that long-term overexpression of AC6 does not alter β-AR signaling except when receptors are activated. This is in contrast to β-AR and G protein overexpression, which cause detrimental effects on cardiac function. The beneficial effect of cardiac-directed expression of AC6 has been reported in Gaq-associated cardiomyopathy and MI model (105, 118). Roth et al. (106) have reported that indirect intracoronary delivery of adenovirus encoding AC6 produces a significant increase in cardiac contractile responses to β-AR stimulation (106). The same group showed that adenovirus-mediated AC6 expression improved cardiac function in cardiac-directed overexpression of Gaq mice (98). In preclinical studies, by using pig pacing-induced HF model, Lai et al. (65) have reported that intracoronary delivery of adenovirus-encoding AC6 attenuates LV remodeling and increases fractional shortening. LV dP/dt max, the minimum values of the first derivative of LV pressure (dP/dt min),

**Fig. 1.** Cardiomyocyte β-adrenergic receptor (β-AR) signaling during heart failure (A) and after treatment with carboxy-terminus of β-AR kinase (β-ARKct) and adenylyl cyclase type 6 (AC6) gene therapy (B). A: G protein-coupled receptor kinase 2 (GRK2) phosphorylates β-AR and facilitates the recruitment of β-arrestin, resulting in β-AR downregulation and impairment of further G protein activation. B: β-ARKct inhibits the recruitment of GRK2, resensitizes β-ARs, and promotes normalization of cAMP signaling. AC6 augments Ca²⁺ 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.
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 sarcosomal 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...
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 Uptregulation 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 intracoronary 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 calcsequestrin-overexpressing mice showed the improvement of cardiac function and Ca2+ transient when crossbred 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 BIO14.6 cardiomyopathic hamsters (49). Intracoronary 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, Wittköpper 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-

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frequency and spontaneous Ca\(^{2+}\) release from the SR, leading to the effects of phosphorylation state in various signaling proteins are required.

\(S100A1\). \(S100A1\) belongs to the \(S100\) protein family, which is the largest EF-hand Ca\(^{2+}\)-binding protein family, and is predominantly expressed in cardiomyocytes, where it is localized in the SR, sarcosome, and mitochondria (84). Myocardial expression levels of \(S100A1\) are decreased in human and nonhuman failing hearts (99, 122). Overexpression of \(S100A1\) transgenic mice showed an augmentation of basal cardiac function in vivo and enhancement of Ca\(^{2+}\) transient in isolated cardiomyocytes that remained to respond to \(\beta\)-adrenergic signal (81). \(S100A1\) transgenic mice subjected to MI did not show HF with preserved global contractile performance and superior survival (83). Adenoviral gene delivery of \(S100A1\) increased contractile function, systolic Ca\(^{2+}\) amplitude and SR Ca\(^{2+}\) uptake, reduced SR Ca\(^{2+}\) leak, and reversed reactivated fetal gene expression in failing cardiomyocytes (8, 80, 82), leading to normalized cardiac function and Ca\(^{2+}\) handling in postinfarcted failing rat heart (82, 93). Recently, Pleger et al. (94) have reported that delivery of \(AAV9-S100A1\) to the LV noninfarcted myocardium in pig chronic MI model prevents and reverses cardiac dysfunction and LV remodeling along with normalized cardiomyocyte Ca\(^{2+}\) cycling and SR Ca\(^{2+}\) handling (94).

Interestingly, the studies of cardiac overexpression of \(S100A1\) suggest that hypercontractile phenotype of transfected cardiomyocytes is independent from \(\beta\)-AR downstream signaling and related protein expression (9, 80, 81). This may be related to the therapeutic effectiveness of \(S100A1\) on HF since chronic stimulation of \(\beta\)-AR signaling and its downstream effectors such as PKA eventually leads to cardiac hypertrophy and HF. The molecular targets of \(S100A1\) are 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 through 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 swine 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-\(\beta\), 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-\(\alpha\) (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 autophosphorylation of JAK-2. JAK-2 then phosphorylates STAT5, PI3K/Akt, and mitogen-activated protein kinase (MAPK). Erythropoietin pre-activates protein kinase C and PI3K/Akt pathway (38). In addition, ATP-dependent and Ca²⁺-activated potassium channels also mediate the protective effects of EPO in ischemic reperfusion injury (13). Recently, it has been reported that EPO induces cardiac mitochondrial biogenesis through EPO receptor-dependent activation of endothelial NO synthase, serving to protect cardiac oxidative phosphorylation during hypoxia (13). EPO promotes angiogenesis by several distinct mechanisms. Westenbrink et al. (129) have reported that EPO stimulates VEGF protein expression through STAT3 pathway predominantly in cardiomyocytes, and secreted VEGF induces the proliferation of endothelial cells and promotes the incorporation of endothelial progenitor cells (129). Ueda et al. (125) have reported that EPO induces angiogenesis by stimulating cardiomyocytes to secrete VEGF and angiopoietin 1 through sonic hedgehog signaling independent of STAT3 pathway. Hochs et al. (48) have reported that STAT3 is crucial for expression of EPO in cardiomyocytes and cardiomyocytes-derived EPO activates blood vessel formation by cardiac progenitor cells through C-C motif ligand-2/C-C motif receptor-2 system (48). Besides protective and angiogenic effects of EPO, it has been recently reported that EPO exhibits direct positive inotropic and lusitropic effects in cardiomyocytes and ventricular muscle preparation through PI3K and PKCε isoform signaling to directly affect both Ca²⁺ release dynamics and myofilament function (45). Figure 3 shows a

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>
</tr>
</thead>
<tbody>
<tr>
<td>Ince et al. (51)</td>
<td>50</td>
<td>50</td>
<td>10 (6)</td>
<td>ECHO</td>
<td>4</td>
<td>LVEF↑, MACE→</td>
</tr>
<tr>
<td>Zohnhöfer et al. (136)</td>
<td>114</td>
<td>59</td>
<td>15 (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>
</tr>
<tr>
<td>Takano et al. (119)</td>
<td>40</td>
<td>62</td>
<td>2.5 (5)</td>
<td>SPECT</td>
<td>6</td>
<td>LVEF↑, MACE→</td>
</tr>
<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.
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>Lipsc et al. (70)</td>
<td>50</td>
<td>50</td>
<td>300 μg (60,000 IU) darbepoetin-α single bolus bIV</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

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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.

REFERENCES


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