REVIEW

Update on gene therapy for myocardial ischaemia and left ventricular systolic dysfunction or heart failure

Thérapie génique pour l’ischémie myocardique et la dysfunction ventriculaire gauche systolique ou l’insuffisance cardiaque

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Summary  Despite considerable advances in pharmacological, surgical and technology-based cardiovascular therapy, left ventricular dysfunction and heart failure are increasingly prevalent health problems. Recent studies suggest that angiogenic gene therapy can restore perfusion in ischaemic myocardial tissue, and that the transfer of nonangiogenic genes may correct defects in calcium handling that contribute to abnormal contractile function in patients with heart failure; however, large clinical trials of gene therapy for treatment of left ventricular dysfunction and heart failure have yet to be completed, and only a small number of genes have been evaluated in patients. Researchers continue to investigate new genes, combinations of genes and

Abbreviations: AAV, adeno-associated virus; CMV, cytomegalovirus; DNA, deoxyribonucleic acid; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; IRES, internal ribosome entry sites; LVD, left ventricular dysfunction; RNA, ribonucleic acid; SDF-1, stromal cell-derived factor-1; SERCA2a, sarcoplasmic reticulum calcium adenosine triphosphatase; Shh, sonic hedgehog; VEGF, vascular endothelial growth factor; CXCL12, chemokine ligand 12; bFGF, basic FGF; AdVEGF-121, adenoviral VEGF-121; AGENT, Angiogenic Gene Therapy; AdFGF-4, adenoviral FGF-4; CUPID, calcium upregulation percutaneous administration of gene therapy in cardiac disease.

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Background

LVD and heart failure are increasingly important health problems despite considerable advances in pharmacological, surgical and technological approaches to treatment. Heart failure is the leading medical cause of hospitalization and is expected to cost the USA health care system $37.2 billion in direct and indirect expenses in 2009 [1]. The most common cause of LVD is coronary artery disease, followed by hypertension and valvular disease. Heart failure, which often develops in patients who survive myocardial infarction, is a debilitating disease with high morbidity and can lead to frequent hospital re-admissions. The prognosis for patients with chronic heart failure remains poor. Nonpharmacological therapies, such as heart transplantation and the use of implantable left ventricular assist devices, are considered appropriate only in later stages of disease progression and, consequently, are considered for a very small fraction of younger patients.

The impaired cardiac contractile function associated with heart failure can be attributed to declines in perfusion and to unfavourable cardiac remodelling. Because gene therapy is designed to restore perfusion, and newer treatments may also target abnormalities in the contractile function of viable cardiomyocytes, it may be considered a promising alternative for the treatment of LVD and heart failure. Here, we describe several genes that are currently under investigation, the advantages and disadvantages of the vectors and delivery routes that are used most frequently, and the implications of findings from preclinical and early clinical trials of cardiovascular gene therapy.

Gene candidates

Numerous proteins, growth factors, cytokines and calcium-transport regulators have been the focus of angiogenic and myogenic research. Several genes have been investigated in vitro and in animal models, and the promising results of these studies have justified the first clinical evaluations. A number of these genes are described below, but only a few have been used clinically.

Vascular endothelial growth factor

VEGF, which could be considered the prototype for angiogenic gene therapy [2], has been shown to improve LVD in animal models of both ischaemic [2] and pacing-induced heart failure [3]. VEGF-A (also called VEGF-1) was first discovered in 1989 [4] and is the founding member of the VEGF protein family. VEGF-A is upregulated during pathological vessel growth [5], but it possesses a diverse array of pro-angiogenic properties and is the isoform used most frequently in gene therapy. Several VEGF-A derivatives, each containing a different number of amino acids (e.g., VEGF-121, VEGF-165, VEGF-189, and VEGF-206), can induce angiogenesis in animal models [6]; their solubilities and binding characteristics (e.g., to heparin and the extracellular matrix) differ, which influences their ability to interact with target cells and, presumably, alters their angiogenic potency. VEGF-165 is the most promising for use in angiogenic therapy, because it is the most potent (100-fold more potent than VEGF-121) and can induce the developmental gradients required to pattern vessel growth [7].

Fibroblast growth factor

The FGF family includes 23 members, of which acidic FGF (FGF-1) and bFGF or FGF-2 are the best characterized. The biological activity of FGF is not well understood. FGF proteins are potent mitogens for a variety of cell types, including endothelial cells, vascular smooth muscle cells and fibroblasts, and FGF secretion stimulates the synthesis of proteases that contribute to angiogenesis by digesting the extracellular matrix. Unlike VEGF, FGF-1 and FGF-2 are not
crucial for embryogenesis, and because they lack a secretory signal sequence, they enter the extracellular space only passively after cell damage. Nevertheless, both have been used successfully to induce angiogenesis [8], and the secreted FGF isoforms, such as FGF-4 and FGF-5, may have even more therapeutic potential.

**Hypoxia-inducible factor**

The metabolic stimuli associated with hypoxia and ischaemia induce expression of a variety of transcription factors that stimulate angiogenesis, and researchers have begun to investigate the genetic transfer of these factors as an alternative to growth-factor-based gene therapy. Because interactions between HIFs and hypoxia response elements trigger an “angiogenesis programme” by upregulating the expression of a number of growth factors and cytokines simultaneously, the therapeutic administration of HIF could enhance vascular growth by mimicking the natural angiogenic response. HIF1-alpha regulates the expression of SDF-1, which is critically involved both in the mobilization of angiogenic progenitor cells from the bone marrow to the peripheral circulation and in the recruitment of mobilized cells to ischaemic tissue [9]. However, HIF1-alpha can also induce cell death and may be significantly less potent than VEGF [10].

**Sonic hedgehog**

Recent experiments performed in our laboratory indicate that the embryonic hedgehog signalling pathway can be reactivated to combat ischaemia in adult mammals. Recombinant Shh protein induces a robust angiogenic effect by upregulating multiple angiogenic factors, including VEGF, in interstitial mesenchymal cells [11], and genetic transfer of Shh enhances the regeneration of ischaemic myocardium by inducing the expression of trophic factors, such as SDF-1, which increases the recruitment and incorporation of bone marrow-derived progenitor cells into the growing vasculature [12]. We have also shown that Gli3, a transcription factor targeted by Shh during hedgehog signalling, is strongly upregulated in the ischaemic tissue of adult mammals and may have a favourable effect on myogenesis and angiogenesis after an ischaemic insult [13]. Because Shh appears to trigger a cascade of pro-angiogenic factors, it may be particularly effective for angiogenic gene therapy.

**Stromal cell-derived factor-1**

SDF-1 (also called CXC CXCL12) is a 68-amino-acid protein of the CXC chemokine family. Two isoforms, SDF-1-alpha and SDF-1-beta, are encoded as splice variants of a single gene and are expressed by both endothelial cells and stem cells. The growth factor activity of SDF-1 has most often been linked to lymphopoiesis and myelopoiesis [14], but interactions between SDF-1 and its receptor CXCR4 also regulate progenitor cell trafficking, and SDF-1-alpha is essential for the recruitment of stem and progenitor cells to ischaemic tissue [15].

**Factors that regulate calcium transport**

Abnormalities in the function of molecules responsible for the rhythmic release and uptake of Ca2+ ions in myocytes contribute to impaired cardiac contractility in patients with heart failure [16], and because myocardial contractility is dependent on ventricular Ca2+ handling, genetic modification of these molecules could be a viable approach for treatment of heart failure. One of the key Ca2+ handling abnormalities in both humans and experimental models of heart failure is caused by a defect in sarcoplasmic reticulum function, and a large body of experimental evidence indicates that SERCA2a plays an important role in the progression of dilated cardiomyopathy. SERCA2a activity is known to decline in late-stage heart failure, and SERCA2a protein and messenger RNA levels are reduced in cardiac tissue isolated from the failing hearts of patients and animals with heart failure [17,18]. Furthermore, gene therapy with a pseudophosphorylated mutant of phospholamban, the principal regulator of SERCA2a, treated cardiomyopathy in hamsters and infarction-induced heart failure in rats successfully for 6 months or more [19,20]. Preclinical studies also indicate that increases in cardiac adenylyl cyclase content improve left ventricular function, attenuate deleterious remodelling and reduce mortality in both heart failure and acute-infarction models. Investigations of adenylyl cyclase type 6 gene transfer have progressed from studies in cultured cardiac myocytes to animal models of heart failure [21].

**Gene delivery**

**Vectors**

Unlike protein administration, gene therapy can lead to high, sustained protein levels; however, the effectiveness of gene transfer depends on the transfection efficiency—the amount of the gene internalized by cells in the target tissue—and the magnitude and endurance of subsequent expression. The cellular insertion and intracellular trafficking of the transgene is facilitated by vectors, which can be categorized as viral or nonviral. Nonviral plasmid vectors were used in early investigations because they are inexpensive, easily constructed, and generally considered safe; plasmid vectors do not initiate inflammation or an immune response and incur no risk of insertional mutagenesis. Plasmid DNA is taken up effectively and expressed by all mammalian cell types, including cardiomyocytes, in vivo; however, the transfection efficiency of plasmid vectors was low in randomized, controlled trials [22,23]. Nevertheless, plasmid vectors could be useful when short-term modification of gene expression may be beneficial, such as immediately after an acute cardiac event, or for initiating mechanisms that lead to progenitor cell recruitment and to the activation of resident stem cells. More recently, small interfering RNAs have become popular for inhibition studies and could provide a new option for nonviral gene manipulation; very high transfection efficiency can be achieved by administering decoy receptors or antibodies that circumvent blocking factors in cardiovascular tissue.

The viral vectors used most frequently for cardiovascular gene therapy are adenoviruses and AAVs; retroviral
vectors were used in early studies, but their popularity has declined because of safety concerns. Compared with plasmid transfection, viral transfection into vessel walls and heart muscle is much more efficient [24], and virally transfected genes are typically expressed for a longer period of time — AAV-transfected genes can be expressed for months — although the duration varies depending on the virus used. In addition, viral vectors typically infect only a limited number of cell types, and this specificity (i.e., tropism) can be advantageous for cardiovascular therapy. Adenoviruses seem particularly effective for transfecting cardiomyocytes [25], and naturally occurring tropisms for vascular smooth muscle cells and cardiomyocytes are among the more useful characteristics of AAVs. However, viral vectors can generate an inflammatory or immunogenic response and may integrate into the cellular genome, which could increase cancer risk. Nevertheless, adenoviruses have a good safety record in cardiovascular clinical trials [22,26], and the likelihood of inflammation, immunogenicity and host-genome integration varies depending on the vector used.

Routes of administration

Clinical acceptance of gene therapy as a routine treatment option will require the development of standardized, practical delivery systems and techniques for delivering the gene to the tissues targeted most frequently, such as the myocardium and blood vessels. Genes can be injected directly into the targeted tissue for treatment of peripheral disease, but local delivery to the heart used to require open-chest surgery or thoracoscopy. Intracoronary administration or catheter-based delivery systems (e.g., navigation and catheter mapping technology) for transcendocardial gene delivery to the myocardium are much less invasive and more feasible for patients with LVD and heart failure [27].

Gene therapy for ischaemic or nonischaemic left ventricular dysfunction and heart failure

To date, most cardiovascular gene therapies are designed to increase vascular growth and perfusion in ischaemic tissue (i.e., therapeutic angiogenesis) [28]; however, declines in heart function can also be attributable to adverse cardiac remodelling, which evolves from a wide variety of biological changes, including the loss of functional cardiomyocytes and disorganization of the contractile response. These abnormalities may be suitable targets for nonangiogenic gene therapies designed to prevent or suppress the development of heart failure (Table 1).

Angiogenic gene therapy

Long-term survival after myocardial infarction is now the most common cause of chronic heart failure, and despite significant medical advances, postischaemic heart failure remains a primary contributor to morbidity and mortality in the western world [29]. One of the primary goals for treating heart failure is improving perfusion in the ischaemic region, thereby preserving functional tissue and (perhaps) restoring function in viable, but dormant (i.e., “hibernating”) myocardium. Several laboratories have demonstrated that therapeutic vascular growth can be achieved in vivo by the genetic transfer of cytokines [2,30]. VEGF and, to a lesser extent, FGF are the most frequently studied and best-developed cytokines used in the clinical setting.

In the REVASC trial [31], 67 patients with coronary artery disease, severe angina and no conventional options for revascularization were randomized to receive direct intramyocardial gene transfer of adenoviral VEGF-121 (AdVEGF-121) via minithoracotomy or to continue receiving maximal medical treatment. Exercise time, the primary efficacy endpoint, was significantly greater in patients treated with AdVEGF-121 than in the control group ($P = 0.026$), and there was no significant difference between the two treatment groups in overall adverse event occurrence. In a phase 1 study, five ‘no-option’ patients with occlusive coronary artery disease and mild LVD received VEGF-165 plasmid DNA (pVEGF-165) via intramyocardial injection after thoracotomy. Patients experienced improvements in collateral vessel growth, myocardial perfusion, myocardial contractile function and clinical status [32]; the same treatment was associated with significant declines in mean ischaemic area in patients with chronic myocardial ischaemia and moderate LVD (mean ejection fraction, 44 ± 4%) [33,34]. In the Euroinject One trial, 80 ‘no-option’ patients with severe, stable, ischaemic heart disease received pVEGF-165 (0.5 mg) or a placebo plasmid; the therapy appeared to be safe and was associated with improved regional wall motion and a favourable anti-ischaemic effect [23], but did not improve significantly stress-induced abnormalities in myocardial perfusion.

The AGENT trials evaluated the intracoronary injection of AdFGF-4 in patients with stable coronary artery disease. Positive trends were observed in the two small phase 1/2

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<th>Table 1</th>
<th>Therapeutic goals and underlying mechanisms for the treatment of left ventricular dysfunction and heart failure.</th>
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<tr>
<td>Therapeutic goal</td>
<td>Mechanisms</td>
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<tr>
<td>Vascularization</td>
<td>Angiogenesis, Vasculogenesis, Arteriogenesis, Lymphangiogenesis, Endothelial function, Endothelial repair, Re-endothelialization, SMC proliferation matrix, Production/degradation, Apoptosis</td>
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<tr>
<td>Cardiomyogenesis</td>
<td>Cardiomyocyte homeostasis, Cellular contraction, Calcium, Hypertrophy, Fibrosis, Apoptosis</td>
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SMC: smooth muscle cell.
Gene therapy and heart failure

Nonangiogenic gene therapy

Although most studies of cardiovascular gene therapy have investigated the use of angiogenic factors, many other genes are potential candidates for the treatment of heart failure. Gene therapy with recombinant AAV vectors coding for the expression of SERCA2a, which declines in patients with heart failure, was well tolerated in both small- and large-scale studies, and restoration of SERCA2a levels to normal levels led to a significant improvement in cardiac function. These findings prompted initiation of the first-in-human phase 1/2 CUPID trial [38]. In the open-label portion of this ongoing study, nine patients with advanced heart failure received a single intracoronary infusion of recombinant AAV SERCA2a; the treatment was associated with an acceptable safety profile and with improvements in a number of symptomatic and functional variables, which supports initiation of the ensuing phase 2, double-blind, placebo-controlled study. A second, randomized, double-blind study (ClinicalTrials.gov identifier: NCT00534703) is investigating the safety and feasibility of SERCA2a gene therapy when delivered with the AAV6 vector and driven by the cytomegalovirus promoter (AAV6-CMV-SERCA2a). Sixteen patients with advanced heart failure who have received a left ventricular assist device will be randomized to receive AAV6-CMV-SERCA2a or placebo infusion into the coronary arteries, and the recovery of contractile function will be assessed during attempts to wean patients from the left ventricular assist device. The results will be assessed in conjunction with two studies in the USA: one of which delivers the same vector via direct injection into the myocardium during left ventricular assist device insertion, and another in which an AAV1-CMV-SERCA2a vector is administered percutaneously.

A substantial amount of data accumulated during the past several years suggests that adenylyl cyclase 6 expression may have unexpected but pronounced favourable effects for the treatment of cardiovascular disease [39]. A clinical study (ClinicalTrials.gov identifier: NCT00787059) is currently underway to determine whether a type 5 adenovirus encoding this gene can be administered safely and is potentially beneficial in patients with congestive heart failure.

Combination therapy

As the characterization of individual gene therapies becomes more complete, preclinical investigations designed to identify the potential complementary or synergistic effects achieved with combinations of therapies have been initiated. The outcomes of these studies will be determined, in part, by the same variables that influence the effectiveness of single-gene therapy, including the model species, the delivery vector, the organ and disease treated, and the genes delivered.

Two (or more) co-injected genes may not be expressed in the intended ratio, because one of the vectors could be preferentially silenced or removed [40]. Thus, therapeutic approaches that rely on combinations of genes will require the development of a gene transfer system that ensures the stable co-expression of both molecules. One system for inducing stable gene expression involves the use of IRESs. IRESs are structural elements located in the 5′ untranslated region of several mRNAs, where they permit the recruitment of translational machinery. These elements can be used to create expression cassettes that code for combinations of genes within a single mRNA sequence (Fig. 1). In a murine hindlimb ischaemia model, Rayssac et al. [41] showed that the expression of FGF-2 and Cyr61 was more stable when both genes were encoded by a single IRES-
DNA that encodes growth factors [45]. These novel factors or DNA that deliver locally high concentrations of angiogenic growth factors have led to the development of pro-angiogenic matrices for either individual treatment, which may improve patient outcomes. Moreover, combined gene-cell therapy may be equally or even more beneficial at smaller doses than those required for either individual approach; further, targeted administration must overcome yet another set of technical limitations. A precise understanding of the mechanisms underlying neovascularization, including the time course and sequential roles of angiogenic and trophic factors, will enable researchers to better mimic the endogenous regenerative response.

**Conclusions**

Results from clinical trials suggest that cardiovascular gene therapy is safe but provides only limited improvements in global cardiovascular variables. However, only a small fraction of potential genetic targets have been investigated in patients, and large clinical trials of gene therapy for treatment of LVD and heart failure have yet to be completed. Furthermore, angiogenic gene therapy appears to restore perfusion in ischaemic myocardial tissue, and the transfer of nonangiogenic genes may correct the defects in calcium handling that contribute to abnormal contractile function. New genes, combinations of genes, expression vectors, delivery systems and approaches that combine gene and cell therapy continue to be developed and tested rigorously; collectively, these refinements promise to improve both patient response and safety.

**Conflict of interest statement**

There are no conflicts of interest.

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**References**


