Current Status and Future Perspectives of Gene Therapy for Heart Failure

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Abstract

Heart failure(HF) is a disease with high morbidity and mortality. The benefits of current pharmacological and device therapy for survival outcomes of patients with HF are limited. Gene therapy represents a novel promising strategy in treating HF, as it can theoretically normalize the aberrantly expressed genes and their regulatory mechanisms permanently. However, the translation of gene therapy for HF from bench to bedside has been less successful. There are many challenges ahead for gene therapy, especially in the areas of selection of the optimal targets, the needs for developing delivery systems and the improvement in design of clinical trials. In this review, we summarize the most promising gene targets which have been used in experimental and clinical studies for treating HF, highlighting the results from several clinical trials. We also review the latest development in gene therapy vectors and delivery methods, aiming to provide directions for future studies.

Introduction

Heart failure(HF) is a global public health problem. There are about 38 million people diagnosed with HF and its prevalence will increase with the ageing of the population.[1] Although development in pharmacological and device therapy have delayed the disease progression and improved survival in patients with HF,[2] patients still suffer from recurrent hospitalizations. Moreover, the financial burden of care for HF is huge. The recent European data showed that 1-year hospitalization rates for acutely hospitalized and chronic HF patients were 43.9% and 31.9%, respectively.[3] In the United States, the total costs for HF are projected to increase from $31 billion in 2012 to $70 billion in 2030.[4] Therefore, it is urgent to explore new strategies to prevent HF. With the development of underlying mechanisms of HF and the transgenic technologies, gene transfer is considered as a novel potential approach for treating HF.

A number of preclinical studies in animal models of HF have suggested benefits of gene transfer in managing HF. However, the translation of gene therapy to clinical trials has been less successful. In this review, we will show the latest developments of targets, vectors, and delivery methods in gene therapy for HF, highlight the results from studies in clinical trials and provide future perspectives for HF by gene transfer.

Targets Employed in Clinical Trials of Gene Transfer

With the increased understanding of the molecular changes in HF, various targets have been identified. Several targets have been employed in clinical trials of gene transfer. The transgenes include Sarcoplasmic/ endoplasmic reticulum calcium ATPase 2a (SERCA2a), adenylyl cyclase 6 (AC6) and stromal cell-derived factor-1 (SDF-1).

SERCA2a

Ca^{2+} mishandling is an important pathophysiological mechanism of HF and is associated with both systolic and diastolic dysfunction of the failing heart.[5]

SERCA2a is a key Ca^{2+} handling protein located in sarcoplasmic reticulum (SR). During cardiac diastole, it plays a role in moving Ca^{2+} from cytoplasm to sarcoplasmic reticulum (SR).[6] Decreased SERCA2a expression and activity are present in myocardium from animal models of HF and patients with HF.[7, 8] Preclinical studies showed that restoration of SERCA2a expression and activity by gene transfer resulted in improvement in cardiac function, reversion of left ventricular remodelling and increases of survival rate.[9, 10] The promising results in preclinical studies lead to CUPID (Calcium Up-regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) trial in 2007. CUPID trial was the first in human trial to test the effects of SERCA2a gene transfer on clinical outcomes of patients with HF.[11]

In the pilot CUPID 1 trial, an adeno-associated viral (AAV1) vector encoding SERCA2a (AAV1.SERCA2a) was used in a small number of patients. Patients treated with AAV1.SERCA2a showed improvements in several efficacy parameters and in clinical outcomes of the patients.[12, 13] Following the promising results, a larger phase IIb, placebo-controlled, double-blind CUPID 2 and two auxiliary studies (AGENT-HF and SERCA-LVAD) were conducted. Unexpectedly, the CUPID 2 trial failed to meet the primary and secondary endpoints and revealed no improvement in the recurrent HF hospitalizations among the patients with HF.[14] Due to the disappointing outcome of the CUPID 2 study, AGENT-HF and SERCA-LVAD were terminated prematurely. In the AGENT-HF, five patients received AAV1.SERCA2a and four patients with placebo at the time of termination. The data showed no significant difference in ventricular remodeling between the AAV1. SERCA2a group and the placebo group.[15] Nonetheless, there was no safety concerns revealed in these studies. The factors which are associated with the negative results remain unclear. Possible causes for the disappointing results could include poor transduction efficiency, insufficient increases in the expression and activity of SERCA2a, inappropriate screening criteria and endpoints and so on. To solve the problems raised in the CUPID and AGENT-HF trials will help success of gene therapy for HF in the future.

AC6

AC6 is part of β-adrenergic signaling, playing a role in converting adenosine triphosphate (ATP) to cyclic

adenosine monophosphate (cAMP) and pyrophosphate.[16] In HF, expression and activity of AC6 are decreased. Transgenic mice overexpressing AC6 showed increased responsiveness of cAMP to β-AR stimulation, normalization of protein kinase A (PKA) activity and improvement in cardiac function.[17,18,19] Adenoviral-mediated AC6 gene transfer was shown to reduce left ventricular remodeling and increase rates of survival in both small and large animal models of HF.[20,21,22]

Recently, Hammond et al. reported the results of a randomized, double-blind, placebo-controlled, phase 2 clinical trial for patients with HF using adenovirus 5 encoding human AC6 (Ad5.hAC6).[23] AC6 gene transfer in patients with HF was demonstrated to be safe and was associated with increases in ejection fraction (EF) and improvement in left ventricular peak -dP/dt at 4 weeks after randomization and a trend towards a reduction in 1-year admission rate. However, the improvements in cardiac function in Ad5.hAC6 treated patients was transient, as increases in EF at 4 weeks was shown to fall off at 12 weeks. In addition, because the study was too small, interpretation of the results was limited. A larger phase 3 clinical trial for AC6 gene therapy is expected to start very recently (ClinicalTrials.gov Identifier: NCT03360448).

SDF-1

SDF-1 gene transfer is another strategy that has been studied in HF.[24, 25] SDF-1 is a factor that plays a role in tissue repair. It induces endogenous stem cells to the injury myocardium, promotes vasculogenesis, inhibit cardiomyocyte death and improve cardiac remodeling.

SDF-1 gene therapy was shown to increase vasculogenesis and improve phase I study using three doses (5, 15, or 30mg) of SDF-1 plasmid treatment for patients with ischemic HF, SDF-1 gene therapy showed potential efficacy.[26] Following the promising results, Stromal Cell-Derived Factor-1 Plasmid Treatment for Patients with Heart Failure (STOP-HF), a Phase II, double-blind, randomized, placebo-controlled trial was conducted to evaluate safety and efficacy of a single administration of SDF-1 delivered via endomyocardial injection in patients with ischaemic HF in 2012.[27] The trial failed to demonstrate amendment in 6 min walk

distance and Minnesota Living with Heart Failure Questionnaire at 4 months after injection. Nonetheless, the results of sub-analysis revealed the potential of SDF-1 gene transfer for improving cardiac function in high-risk ischaemic HF at 1 year after SDF-1 administration. Currently, retrograde infusion of SDF-1 via the coronary sinus is being studied to see the effects of SDF-1 gene transfer on quality of life measure in patients with ischemic HF in RETRO-HF (ClinicalTrials.gov Identifier: NCT01961726).

Other Promising Targets Studied in Large Animal Studies

Apart from SERCA2a, targeting phospholamban (PLN) and S100A1 are among the most promising strategies for improving Ca^{2+}-handling in gene therapy for HF. PLN plays a role in inhibiting the affinity of SERCA2a to Ca^{2+}. Phosphorylation of PLN relieves its inhibitory effects.[28] During HF, the activity of protein phosphatase-1 (PP1) is elevated, resulting in PLN dephosphorylation and subsequently decreased Ca^{2+} uptake via SERCA2a. Inhibition of PP1 through overexpressing its inhibitor (inhibitor-1) was shown to increase PLN phosphorylation and improve cardiac function in animal models of HF.[29, 30] Delivery of a mutant form of PLN was capable of reducing the inhibitory effects of PLN and improving cardiac function.[31, 32]

S100A1 is a small protein, belonging to a family of Ca^{2+}-regulated proteins. It enhances systolic and diastolic function of the heart through regulating SERCA2A/PLN and ryanodine receptor (RyR) function. S100A1 was found to be reduced in HF.[33] S100A1 gene transfer were able to normalize cardiac function and improve cardiac energetic metabolism both in small and large animal models of HF, implying that S100A1 may be a promising therapeutic transgene for HF.[34, 35]

Another target is involved in the β-adrenergic system. In HF, downregulation and desensitization of β-adrenergic receptor (βAR) occur partly due to increased G-protein-coupled receptor kinase 2 (GRK2) activity.[36] Inhibiting GRK2 activity represents an effective method to reverse βAR desensitization. Transduction of a peptide inhibitor of GRK2 (βARKct) have been shown to improve the cardiac contractile function and reverse ventricular remodeling both in vitro[37] and in large animal

models of HF.[38]The promising results indicate that βARKct overexpression is a potential therapeutic target in HF.

Gene Delivery System

Apart from selecting the right targets, efficient gene delivery system is another assurance of successful gene therapy. The main components of gene delivery system are the vectors and delivery methods, which have been studied for many years to increase efficacy of transduction.

Delivery Methods

The delivery methods that have been used in clinical trials of cardiovascular gene therapy mainly include direct intramyocardial injection and peripheral intravenous injection.[39,40]

Intramyocardial injection can be accomplished by using catheter techniques or during open-heart surgery. It has been widely used both in animal studies[41]and clinical trials.[27] For example, in STOP-HF, intramyocardial injection was applied to deliver a DNA plasmid expressing SDF-1 into peri-infarct border zones of patients with HF. The advantage of intramyocardial injection is that it directly introduces transgenes into the myocardium and hence bypasses the endothelial barrier, resulting in high transgene expression at the injection site. In addition, it also avoids the problem of vector exposure to off target organs.[42] However, the target area of intramyocardial injection is local and the transgene expression is often inhomogeneous, leading to limited application of this delivery method for HF.[43, 44]

Peripheral intravenous injection includes antegrade intracoronary injection and retrograde injection through the coronary sinus into the venous system of the heart. Retrograde injection will increase exposure time and subsequently improve the transduction efficacy.[45] However, this method requires occlusion of the coronary artery and the sinus to block antegrade flow. Long-time occlusion may increase rates of delivery-related complications and lead to intolerance by the patients with HF.

The techniques employed in the antegrade intracoronary injection is similar with that used in

percutaneous coronary interventions, which is safer and easier. In addition, the method allows for homogeneous distribution of vectors. However, the disadvantage of antegrade delivery is that it often results in relatively low vector transfection efficiency. Myocardial uptake is influenced by virus concentration, exposure time to the virus, coronary flow rate, vascular permeability, perfusion pressure and so on.[46] Efforts to improve the transduction efficiency of intracoronary delivery of gene therapy have been made in clinical trials. For example, Nitroglycerin (NTG) was used to increase vascular permeability and improve vectors uptake in the CUPID 2 trial[47] as NTG was demonstrated to have the effects of increasing AAV1/SERCA2a myocardial transduction efficiency in preclinical studies.[48]

Vectors

Various vectors have been developed for gene transfer in fundamental and clinical studies. Each kind of the vector has its own advantages and disadvantages. Selecting the right vector is an important element for the success of gene therapy.

Vehicles used in cardiovascular gene therapy mainly include non-viral vectors and viral vectors. To date, non-viral gene vectors often refer to naked plasmid DNA, which is easy to produce and has no limitation of DNA size. Furthermore, naked plasmid DNA also has the advantage of lack of a significant immune response, resulting in low biosafety risk.[49]As aforementioned, in the STOP-HF trial,[27] naked plasmid DNA was used to deliver SDF-1 into the peri-infarct zones of patients with HF, aiming at recruiting stem cells in a short period of time. However, naked plasmid DNA may be not suitable for delivering the majority of targets in HF as it has the limitation of low transfection efficiency, leading to a transient effect. Therefore, further studies are needed to improve the transfection efficiency of naked plasmid DNA.

As the limitations of non-viral vectors, the majority of studies targeting HF have applied viral vectors, which are able to provide higher transduction efficiency of the transgene than non-viral vectors. Over the past years, great efforts have been made to improve the capacity of virus to deliver genes to the target organs. Various kinds of viral vectors have been used for gene therapy, among

which adenovirus, adeno-associated virus (AAV), and lentivirus vectors have been the interests of researchers.

Adenovirus can be produced in large quantity and are capable of delivering materials to almost all kinds of cardiac cell types efficiently.[50] They are widely used in gene therapy for cardiovascular disease in preclinical studies.

However, the application of adenovirus in clinical trials is limited. Adenoviral mediated transgene expression is transient, only with a duration of expression from days to 2 weeks.[51] Another major disadvantage of adenovirus is that they evoke immune and inflammatory reactions, raising question about safety of administration in patients.[52, 53]

Lentiviral vectors can integrate into the host genome and are able to provide long-term transgene expression both in animals and human.[54-56] However, the application of retroviral vectors is limited in cardiovascular disease due to their low specific tropism for cardiomyocytes. Novel virus type has been developed to overcome the obstacle in experimental studies.[57]

AAV is a nonpathogenic human virus and was first discovered in adenovirus preparations, belonging to the parvoviridae family.[58] AAVs are currently the vector of choice in numerous clinical trials of gene therapy.[59] They can not only provide stable long-term gene expression, but also show excellent safety when transduce various types of cells. Importantly, among the currently recognized 13 AAV serotypes, AAV1, AAV6, AAV8 and AAV9 have been demonstrated to be highly cardiotropic so that the use of AAV in cardiovascular disease is much concerned.[60] However, the existence of neutralizing antibodies resulted from prior infection in human populations limited the application of AAV vectors. In the CUPID 2 trial, about 60% of patients were excluded because of the preexistence of AAV1 neutralizing antibodies in their serum.[61] Recently, attempts have been made to minimize the negative effects of neutralizing antibodies in fundamental studies.[30,62]

Conclusions and Future Perspectives

There is a great demand to develop novel strategies for treating HF. Gene therapy is among the most promising approaches of reversing the fundamental abnormalities in failing cardiomyocytes. However, though a majority of preclinical studies have demonstrated promising results, the successful experience of translating gene therapy for HF to clinical trials is absent. Detailed analysis of the completed clinical trials will help improve gene therapy strategies.

In the CUPID 2 trial, the amount of vector DNA within the available myocardium from 7 patients were an approximate median of 43 copies per µg DNA, representing the lower end of the threshold for dose-response curves (<500 copies per µg DNA) in pharmacology studies.[14] It seems that insufficient transduction efficiency is a major problem in gene therapy. To increase transduction efficiency, there is a need to select the best strain of AAVs, optimize the dose of drug, develop more efficient gene delivery methods and eliminate the effects of antibodies in future. In addition, identifying endogenous factors that may interact with vectors and the therapeutic effects in each strategy is also needed.

Another challenge is that there is currently no uniform strategy for selecting patients and endpoints, which are pivotal to the success of gene therapy.

In many clinical trials of gene therapy, patients with advanced HF are often screened. However, gene therapy may be effective in only some subgroups of patients. It is urgent to develop methods to select the most suitable patients for each clinical trial. Various endpoints (e.g. survival, exercise tolerance, biomarkers, recurrent HF hospitalizations) have been used in previous clinical trials. There is a clear need to develop validated endpoints in future clinical trials.

Finally, current clinical trials of gene therapy for HF have shown no safety concerns. This should encourage the conduction of more clinical trials to test therapeutic effects of new targets and improved gene delivery systems.

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