

Chapter 15

Gene Therapy in Cardiovascular Disease

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Veritas filia temporis – Aulus Gellius

Abstract Cardiovascular gene therapy applications began about 25 years ago. Since then, an in-depth understanding has accumulated on the underlying mechanisms of molecular structure as well as the development and function of the cardiovascular system in normal and disease states. In accordance with this, gene-based approaches have undergone substantial changes. Cardiovascular gene therapy should ideally deliver the genetic material to a specific target and reach a level of expression sufficient for therapeutic action. To achieve this, one needs to select a strategy with gene overexpression or gene silencing, suitable vectors and promoters, specific molecular targets known to be involved in a certain cardiovascular disease, and organ-targeted delivery techniques. Pharmacologic intervention has substantially increased survival and decreased morbidity in acquired and congenital cardiovascular diseases but still has multiple limitations including the targeting of symptoms rather than the pathological mechanism, difficulty in achieving efficacy, large variation between dose and concentration-dependent pharmacokinetics, and side effects. The progress in molecular biology and pharmacogenomics technology could allow for the development of gene containing drugs, which have the potential in the near future to momentarily improve the management of a variety of clinical cardiovascular problems.

Keywords Gene delivery vectors • Gene transfer techniques • Cardiovascular molecular targets • Cardiovascular gene therapy • Coronary heart disease • Heart failure

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15.1 Introduction

Currently, gene-based therapy is recognized as a potentially powerful new therapeutic weapon for treating recurrent and/or refractory cardiovascular disease. Genetic manipulation may supplement or be applied without standard pharmacotherapy. Advantages of gene therapy approaches over traditional treatment include the ability to change the structure and function of the cell at the molecular level and to directly target intracellular signaling pathways. These effects can lead to cellular reprogramming with tissue regeneration that cannot be accomplished with existing drugs. Moreover, gene therapy provides the possibility to maintain a constant high concentration of transgene in desired tissues or organs over time, with a potential for more favorable cost because it does not require daily use for chronic disease. Currently, it is unclear if and how transgene expression could be terminated after exerting its therapeutic effect.

During the last decade, the concept and purposes of gene therapy have changed considerably. Today, it includes not only replacement of a defective gene with a functional copy but also use of nucleic acid transfer to treat or prevent complex multigenic diseases. Moreover, the earlier objectives were only to cure diseases not amenable to regular pharmacotherapy; gene therapy now includes viral and nonviral transfer of different genes to treat the majority of cardiovascular disease, genetic modification of stem cells for transplantation, delivery of antisense technology or ribozyme RNA to inactivate a specific gene at the mRNA level, vaccination with transgene inducing expression of antigens for immunization, sequence-specific gene silencing with short hairpin RNA, etc.

At the beginning of the era of gene transfer, researchers perceived it only as a tool to explore new genes and their functions, to study signal transduction and regulation, and to develop transgenic animal models. Creation of vectors for the insertion of genes into mammalian cells and the decoding of the human genome opened the door for the clinical use of gene-based treatment. As expected, the development of such a treatment has encountered many challenges and uncovered previously unknown insights into gene interactions and regulation.

The first cardiovascular gene transfer was demonstrated in 1989 with the transfection of porcine endothelial cells *ex vivo* with retrovirus carrying a marker gene [1]. The first clinical trial was performed later using direct introduction of a VEGF gene construct as naked DNA to treat hind limb ischemia [2]. Recently, the first gene therapy drug (Glybera) was approved by the European Medicines Agency for the treatment of lipoprotein lipase deficiency [3]. Despite this progress, we must note with regret that the number of cardiovascular clinical trials as of 2014 is 162 compared to 1,331 for cancer, placing fourth among gene therapy clinical trial targets in spite of the fact that cardiovascular disease is still the leading cause of death in the world [4] (Fig. 15.1a).

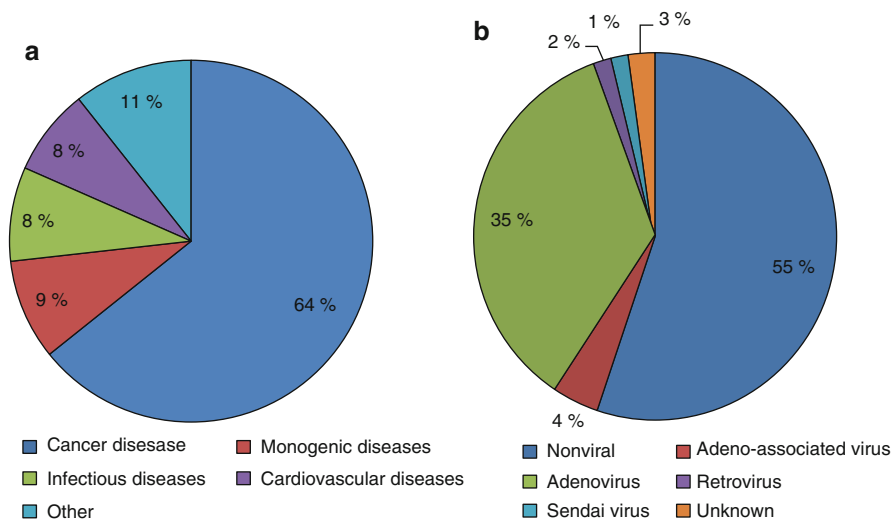


Fig. 15.1 Gene therapy clinical trial data. (a) Relative distribution of clinical trials by disease. (b) Relative distribution of cardiovascular gene therapy clinical trials by vector used

15.2 Gene Therapy Strategy

Two basic types of gene therapy strategies are currently employed in cardiovascular disease.

The *first strategy* is the exogenous overexpression of a target gene, aiming to increase the activity of a gene whose endogenous function may be impaired or downregulated as a result of mutation or a pathological process. In this case, cDNA encoding the deficient gene is delivered to the nuclei and the replaced gene product is expressed and interacts with a defined cell mechanism. The goal is to restore normal function or reverse disease progression.

The *second strategy* is the inactivation or silencing of target genes exhibiting maladaptive activity. Potential approaches in this strategy include (i) expression of a peptide or protein inhibitor, (ii) the use of truncated proteins to express only the part of the protein aimed at direct inhibition of enzyme function or disruption of protein-protein interactions, and (iii) the use of RNA interference technology [5]. This strategy can be performed at the transcriptional level (antisense oligonucleotides) or at the posttranscriptional level (ribozymes and small interfering RNA).

- Double-stranded oligonucleotides (decoy) have been used to inhibit transcription factors involved in the activation of pathogenic genes [6].
- Short single-stranded deoxyoligonucleotides (antisense oligonucleotides) bind to the target gene mRNA and prevent it from being translated.

- Small interfering RNAs (siRNAs) are short RNAs that knock down the endogenous activity of a pathogenic gene by induction of sequence-specific gene modification.
- Proof of principle of efficient RNA interference using adenovirus-based vector was demonstrated in the phenotyping of cardiac myocytes [7].
- Ribozymes are used to degrade target mRNA transcripts copied from the gene. Selective blockade with ribozyme oligonucleotides in a rat model resulted in inhibition of neointimal formation after vascular injury [8].

15.2.1 Ex Vivo and In Vivo Gene Therapy

Ex vivo gene therapy involves the harvest of cells from a patient followed by therapeutic gene replacement or addition into the target cell genome via vector. After this transduction, the cells are returned to the patient to exert their therapeutic effect. As an example, a study on VEGF was performed in a murine model. After 7 days in culture (ex vivo), endothelial progenitor cells (EPCs) were transduced with an adenovirus encoding the VEGF 164 gene. This manipulation augmented EPC proliferative activity and enhanced adhesion and incorporation of EPCs into quiescent and activated endothelial cells. After that, gene-modified EPCs were administered to mice with hind limb ischemia. Neovascularization and blood flow recovery were both improved, and limb necrosis was reduced by 63.7 % compared to control animals [9]. This ex vivo technique can allow for increased transgene expression, but can only be applied to certain cell types. Conversely, in vivo gene therapy involves the introduction of a virus carrying the genetic material directly into body tissue.

15.3 Main Prerequisites for Successful Cardiovascular Gene Therapy

Achieving specificity and efficiency of cardiovascular gene therapy requires the application and interaction of several essential factors:

- (i) Selection of appropriate transfer vector and promoter
- (ii) Development of targeted route and technique of gene delivery
- (iii) Validation of the correct transgene molecular targets

Genetic material must be transferred into cells and expressed either at a constant level with insertion of the DNA into the cell genome or on a temporary basis with preservation of the DNA in an episomal state. Only use of special vehicles called vectors results in sufficient transport of genetic information into a cell. Vectors can be divided into viral and nonviral delivery systems. The optimal vector should be safe, have the ability to be transduced in vivo or ex vivo with reinfusion to the

patient, restrict expression to the desired tissue, provide the desired longevity of expression, have sufficient capacity for the genetic material to be transferred, and minimize the risk of an immune response.

15.3.1 *Nonviral Vectors*

Nonviral gene therapy targeting the cardiovascular system began to develop before viral vectors and is still the most prevalent approach in clinical trials (Fig. 15.1b). The basic advantages of nonviral vectors include a relative lack of inflammatory and immune responses (allowing gene reintroduction) as well as low toxicity and the absence of a potential for mutagenesis, removing the safety concerns characteristic of viruses. Moreover, there is no limiting size for transgene and both ease of production and stability over time [10]. The materials used in nonviral cardiovascular applications include plasmid DNA (pDNA) and small nucleic acids (antisense oligonucleotides or small interfering RNAs). pDNA is a double-stranded DNA encoding the gene of interest. The most significant limitation of nonviral vectors is a low transfer efficiency resulting in poor transgene expression. To improve this, many investigators have begun using chemical-based vectors such as cationic lipids and cationic polymers which promote cell uptake and trafficking and protect DNA from intracellular degradation. Left ventricular injection of pDNA in vivo was first performed about 25 years ago. Marker gene expression was observed in myocytes 3–4 weeks after delivery [11]. By 1998, a phase I clinical trial demonstrated the safety and efficiency of gene transfer of pDNA encoding vascular endothelial growth factor (VEGF) in patients with thromboangiitis obliterans (Buerger's disease) [12]. The same year, naked pDNA encoding VEGF was used as a sole therapy for patients with symptomatic ischemic heart disease (IHD). All patients had reduction in angina and improvement of myocardial perfusion on coronary angiography [13]. In another clinical trial, 28 patients with coronary heart disease (angina class II–III) received catheter-based intracoronary delivery of VEGF-plasmid liposome. This study demonstrated the safety and feasibility of liposome-mediated gene transfer [14].

15.3.2 *Viral Vectors*

Genetic engineering of vector from virus requires that coding genes and *cis*-acting sequences be separated into distinct nucleic acid molecules to prevent their reconstitution by recombination into productive viral particles. Linking of the viral *cis-acting* sequences (noncoding DNA regulating gene transcription) to the therapeutic gene produces replication-deficient particles able to transfer new genetic information [15]. The majority of viral vectors used for cardiovascular applications were derived from human pathogens from which essential genes have been deleted.

Table 15.1 Available vector systems for cardiovascular gene therapy

I. Nonviral	
<i>Advantages</i>	<i>Limitations</i>
<i>(a) Naked pDNA</i>	
Simple methodology	Low transduction efficiency
No limit to transgene size	Transient gene expression
Little immunogenicity and oncogenicity	
<i>(b) Antisense oligonucleotides</i>	
Easy to produce	Limited efficiency
No immune response	Degradation by nucleases
No integration in host cell genome	Transient effect
II. Viral	
<i>Advantages</i>	<i>Limitations</i>
<i>(a) Adenovirus</i>	
High transgene capacity	Immune-inflammatory response
High transduction efficiency	Short-term expression
Transfer to cardiac and vascular cells	
<i>(b) Adeno-associated virus</i>	
Long-term gene expression	Limited transgene capacity
Low immunogenicity	Difficult to produce in large quantities
High tropism to cardiac and vascular cells	
<i>(c) Lentivirus</i>	
Long-term expression	Integration into the host cell genome
Low immune response	Limited cardiovascular tropism
	Risk of oncogenicity

Several viral vectors have been explored successfully for cardiovascular gene transfer because of their various advantages (Table 15.1).

15.3.2.1 Lentiviral Vectors

These vectors are derived from primate and nonprimate immunodeficiency viruses. Expression and therapy are sustained and induce nonsignificant immune response. Maintenance of uncompromised cellular function and gene transfer to nondividing cells make these vectors attractive for a wide range of disease targets including cardiovascular diseases. It was shown that lentivirus-based vectors can effectively transduce well-differentiated cardiac myocytes and fibroblasts [16]. Third-generation lentiviruses with a majority of their native genome deleted can transduce human saphenous vein endothelial cells and smooth muscle cells (SMC) better than adeno-associated virus (AAV) serotypes [17]. The major limitation of lentiviral vectors is the risk of mutagenesis and oncogenesis.

15.3.3 Adenoviral Vectors

Adenoviruses contain a double-stranded DNA genome which remains episomal after introduction. Although this type of vector has historically been the most commonly used for preclinical and clinical studies in cardiovascular gene therapy, the pendulum is swinging toward the use of adeno-associated virus for many of these applications. The prevalent use of this vector is due to high expression kinetics, large cloning capacity, broad target cell tropism, efficient levels of transgene expression, and ease of high-titer manufacturing. Porcine hearts infected with an adenovirus vector containing the β -galactosidase (β -gal) gene showed significantly increased β -gal enzymatic activity compared to hearts injected with β -gal plasmid, with the efficiency of adenovirus-mediated gene transfer 140,000 times superior to plasmid DNA injection [18]. Clinical trials using recombinant adenoviral vectors to deliver angiogenic growth factors demonstrated therapeutic benefit [19]. However, adenoviral vector disadvantages such as innate and adaptive immune responses, transient gene expression, and a propensity to trigger inflammatory and toxic reactions in the host undoubtedly restrict its use.

15.3.3.1 Adeno-associated Viral Vectors

Lack of human pathology, low immunogenicity, strong tissue tropism for the heart and vessels, and efficient transduction of cardiomyocytes and SMC make this vector very attractive for cardiovascular applications. AAV can transfer a single-stranded DNA only about 20–25 nm (4.7 kb) in size. At least 11 AAV serotypes have already been described and a much larger number can be engineered through recombination of existing AAV viral capsid sequences [20]. The development of new AAV capsids significantly promoted AAV gene transfer technology in the last decade. Efficient transduction and persistent transgene expression in cardiac tissue were demonstrated in different animal models [21]. The ability of various AAV serotypes to transduce vascular cells *in vitro* and *in vivo* has also been proven [22]. Major concerns about AAV vector are its small packaging capacity, difficult production of high-titer vector stocks, and presence of preexistent neutralizing antibodies in at least 50 % of the human population.

15.4 Gene Delivery Techniques for Cardiovascular Applications

A great number of gene delivery techniques have been identified since the onset of cardiovascular gene therapy (Fig. 15.2). Two major conclusions should be drawn from the data: the route of gene delivery is no less important than the vector system, and gene delivery should be organ targeted with minimal or optimally zero collateral expression.

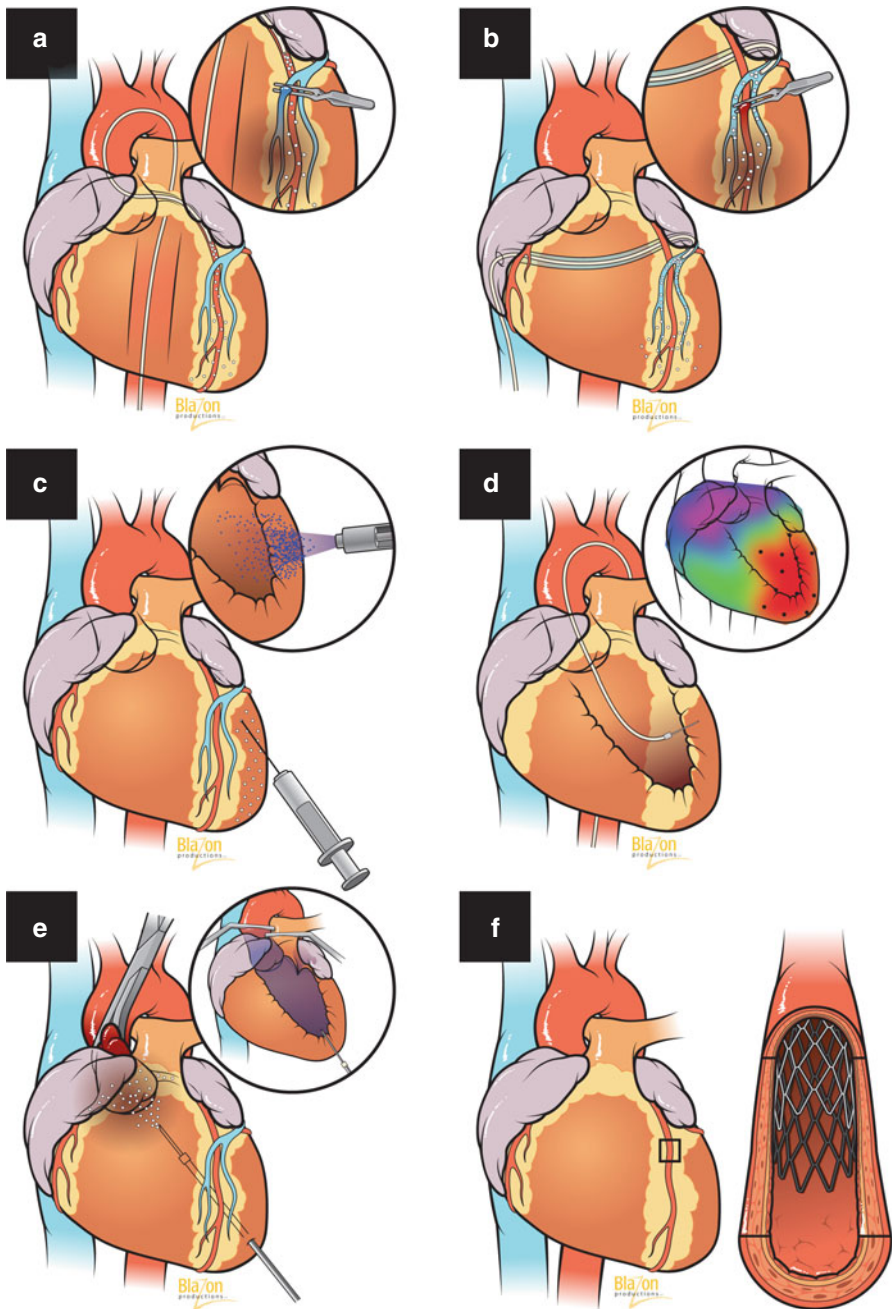


Fig. 15.2 Gene therapy delivery methods. **(a)** Antegrade intracoronary delivery (*inset*: with venous blockade). **(b)** Retrograde transcortical sinus (*inset*: with arterial occlusion). **(c)** Intramyocardial injection, epicardial approach (*inset*: liquid jet injection). **(d)** Intramyocardial, endocardial approach (*inset*: image-guided delivery). **(e)** Intracavitary injection to left ventricle with aortic occlusion (*inset*: with aortic and pulmonary artery occlusion). **(f)** Transvascular intracoronary wall delivery via gene-eluting stent (*inset*: stent within artery)

15.4.1 Transvascular Route

Intravenous administration is the least invasive and simplest route for gene transfer. It finds its application in the treatment of diseases such as systemic hypertension and hyperlipidemia. However, in peripheral arterial disease as well as acquired and congenital cardiac disorders, this technique is ineffective due to first-pass pulmonary and hepatic uptake of vector and systemic dilution in blood circulation. Efficacy of antegrade intracoronary administration is much better, although it cannot escape systemic leakage. In an effort to achieve increased transduction, researchers began to use concomitant coronary venous blockade [23], transient coronary occlusion [24], and increased perfusion pressure [25]. Retrograde gene delivery through the coronary sinus enhanced expression due to a more than tenfold increase in coronary passage time [26] and an increase in venous capillary filtration rate [27]. Another significant advancement was the creation of a *closed-loop recirculatory system* which allowed separate heart and systemic circulation [28, 29]. Gene-eluting stents for *localized transvascular wall delivery* in cardiovascular pathology represent a new attractive alternative to standard angioplasty and vascular interventions. However, this method still needs additional research to assess its transduction efficiency.

15.4.2 Direct Intramyocardial Delivery Using Mechanical and Physical Approaches

Unlike the transvascular route, during direct gene delivery, transgene enters the extracellular matrix and somatic cells, bypassing the blood compartment which includes plasma proteins, blood cells, and neutralizing antibodies which substantially inactivate the vector. Moreover, this method allows for the application of high concentrations of transgene directly at the target site. This approach has been successfully applied in animal models of ischemia and cardiac arrhythmias as well as several clinical trials to induce angiogenesis [30]. Relatively low transduction efficiency led to use of image guidance devices such as the Noga system and a variety of physical and mechanical approaches enhancing cell membrane permeability for gene transfer. The most commonly used are *electroporation* which involves high-intensity electric pulses, *sonoporation* which involves attachment of genes to gas-filled microbubbles which are destroyed by ultrasound after injection, use of energy sources such as *laser* to induce tiny holes in cell membranes, and transfer of gene nanoparticles under the influence of a *magnetic field*. *Liquid jet injection*, *gene gun particle bombardment*, and *microinjection* are additional mechanical methods which have found application in direct gene delivery for cardiovascular disease.

15.5 Molecular Targets

Identification of potential targets in cardiovascular disease is a very complex and laborious process closely associated with the development of basic science in molecular biology and genetic engineering. For each new target, it is necessary to determine signaling pathways, cell membrane receptors, transcription factors, intracellular trafficking, and many other items that make it possible to influence them through gene therapy (Table 15.2).

15.5.1 Coronary Heart Disease (CHD)

CHD alone causes one of every six deaths in the United States. In 2014, an estimated 620,000 Americans will have a new coronary attack (defined as first hospitalized myocardial infarction or CHD death) and 295,000 will have a recurrent attack [31]. The failure rate of interventional coronary revascularization as a result of restenosis, multiple stenotic lesions, or suboptimal anatomy remains relatively high. Therefore, the possibility that a one-time delivery of transgene to myocardium may induce global therapeutic angiogenesis or improve contractile function appears very attractive. For CHD, the current genetic targets are varied (Fig. 15.3). It is quite likely that in the future, gene products must act on multiple molecular pathways or supplement existing pharmacotherapy of revascularization procedures.

A vast amount of preclinical and clinical research including phase I–II/III trials has been published regarding angiogenesis [32]. All studies demonstrated excellent safety and feasibility of using recombinant growth factors in CHD [33]. Preclinical gene therapy studies with vascular endothelial growth factor (VEGF) in various animal models of myocardial ischemia have demonstrated improvement in contractility and reduction of both infarct size and peri-infarct fibrosis [34, 35]. Although there was no clear demonstration of a clinical benefit in patients, important factors affecting efficient gene transfer in CHD were identified [36]. The exogenous administration of angiogenic factors including VEGF, hepatocyte growth factor, fibroblast growth factor (FGF), hypoxia-inducible factor-1 α (HIF), angiopoietin-1, and insulin-like growth factor is a reasonable approach for therapeutic neovascularization. A phase II KAT trial provided evidence that VEGF-165 gene therapy during percutaneous coronary intervention increased myocardial perfusion [14]. The AGENT study with symptomatic CAD showed that after adenovirus-mediated FGF gene transfer, ischemic defect decreased in patients who were not candidates for revascularization [37]. In the REVASC clinical trial, adenovirus containing VEGF-121 was delivered by direct intramyocardial injection. Administration of VEGF121 resulted in objective improvement in exercise-induced myocardial ischemia [38]. A multicenter, randomized, double-blind, placebo-controlled clinical trial using HIF demonstrated improved perfusion by positron emission tomography analysis [39]. It is not surprising that animal experiments on healthy subjects without comorbidities using recombinant growth factors for angiogenesis were very promising yet the

Table 15.2 Gene therapy targets in cardiovascular disease

Cardiovascular diseases	Aims	Target genes
Hyperlipidemia	1. Reduction of LDL cholesterol	ApoB, apoE, apoA-1, eNOS, LDL receptor, NF-κB, VLDL receptors, decoy MSR, TNF-α, TIMPs
	2. Target atherosclerotic lesions	
Systemic hypertension	3. Modulation of MSR activity	Kallikrein, adrenomedullin, eNOS atrial natriuretic peptide, angiotensinogen, β-adrenergic receptor, ACE, angiotensin receptor
	1. Inhibition of genes involved in BP elevation	
Pulmonary hypertension	2. Inhibition of vasoconstriction-promoting genes	BMPR2, eNOS, CGRP, prostacyclin synthase, adrenomedullin, VEGF, HGF
	1. Suppression of pulmonary SMC proliferation and differentiation	
Heart failure	2. Inhibition of pulmonary vascular remodeling	SERCA2a, S100A1, phospholamban, PP-1, βARs, βARKct, adenylyl cyclase, Bcl-2, P13, Akt, HSP, TNF-α, HO-1, SDF, angiotensin II, endothelin 1, TIMP-1, parvalbumin, TGF-β system
	1. Enhancing excitation-contraction coupling	
	2. Reduction of adverse remodeling	
	3. Inhibition of apoptosis	
	4. Abrogation of fibrosis	
Cardiac arrhythmias	5. Cytoprotection, stem cell repair	Gαi2, KCNE3, connexin-43, KiR(2.1b), TGF-β, βARs, HCN2, Kv1.5
	1. Heart rate control	
Peripheral arterial disease	2. Biological pacemaker function	VEGF, HGF, FGF, HIF-1α, Del-1, PDECGF, eNOS, TIMPs, HO-1, prostacyclin, SCDF-1α, sonic hedgehog, netrin, thrombospondin, C-type natriuretic peptide, COX, Ras, Fas ligand, β-interferon, βAR kinase
	3. Repolarization and reduction of QT interval	
	4. Modulation of cardiac conduction	
	1. Therapeutic angiogenesis	
	2. Stabilizing plaque and diminishing risk of rupture	
	3. Prevention of thrombosis	apoB apolipoprotein B, apoE apolipoprotein E, apoA-I apolipoprotein A-1, eNOS endothelial nitric oxide synthase, LDL low-density lipoprotein, NF-κB nuclear factor kappa-light-chain enhancer of activated B cells, VLDL very low-density lipoprotein, MSR macrophage scavenger receptor, TNF-α tumor necrosis factor-α, TIMP tissue inhibitor of metalloproteinase, ACE angiotensin-converting enzyme, BMPR2 bone morphogenetic protein receptor type II, CGRP calcitonin gene-related peptide, VEGF vascular endothelial growth factor, HGF hepatocyte growth factor, SERCA2a sarco(endo)plasmic reticulum Ca ²⁺ -ATPase isoform 2a, S100A1 S100 calcium-binding protein A1, PP-1 protein phosphatase-1, βARs β-adrenoreceptors, βARKct β-adrenergic receptor kinase-1 (carboxy terminus), Bcl-2 B-cell lymphoma 2 gene, P13 p13 tumor suppressor gene, Akt protein kinase B, HSP heat shock protein, HO-1 heme oxygenase-1, SDF stromal cell-derived factor, TGF-β transforming growth factor β, Gαi2 G-protein i subunit α type 2, KCNE3 potassium voltage-gated channel Isk-related family member 3, KiR(2.1b) human cardiac inwardly-rectifying K+ channel 2.1b, HCN2 potassium/sodium hyperpolarization-activated cyclic nucleotide-gated ion channel 2, Kv1.5 voltage dependent potassium channel Kv1.5, FGF fibroblast growth factor, HIF-1α hypoxia-inducible factor 1-α, Del-1 developmental endothelial locus-1, PDECGF platelet-derived endothelial cell growth factor, SCDF-1α stromal cell derived factor-1α, COX cytochrome c oxidase, Ras rat sarcoma G-protein superfamily, Fas tumor necrosis factor receptor subfamily 6
	4. Limit restenosis after angioplasty	
	5. Hindering intimal hyperplasia in vein grafts	

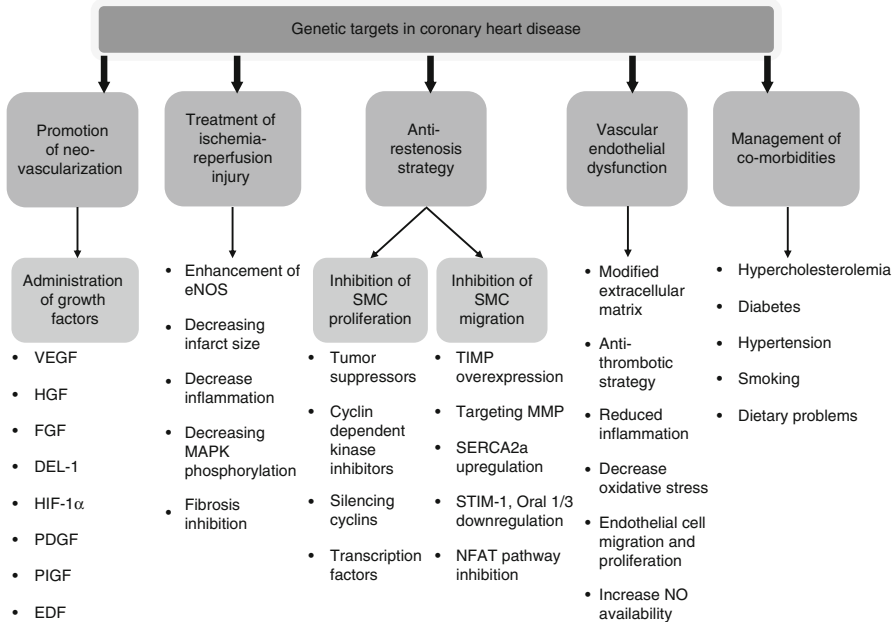


Fig. 15.3 Genetic targets in coronary heart disease. *VEGF* vascular endothelial growth factor, *HGF* hepatocyte growth factor, *FGF* fibroblast growth factor, *DEL-1* developmental endothelial locus-1, *HIF-1* hypoxia-inducible factor-1, *PDGF* platelet-derived growth factor, *PIGF* placental growth factor, *EDF* erythroid differentiation factor, *eNOS* endothelial nitric oxide synthase, *MAPK* mitogen-activated protein kinase, *SMCs* smooth muscle cells, *TIMPs* tissue inhibitor metalloproteinases, *MMPs* matrix metalloproteinases, *SERCA2a* sarcoendoplasmic reticulum calcium adenosine triphosphatase isoform 2a, *SIM-1* stromal interaction molecule-1, *Oral 1/3* ORAI calcium release-activated calcium modulator 1/3, *NFAT* nuclear factor of activated T cells, *NO* nitric oxide

clinical studies demonstrated limited benefits. Furthermore, most of the clinical trials in CHD involved the use of gene therapy for “no-option” patients (patients whose cardiovascular health is poor enough to preclude any other treatments). With these conditions limiting the effectiveness of gene therapy, a single delivery may not cause measurable improvement. Thus, it is very important to choose patients who may respond to gene therapy treatment and to standardize the stage of disease, pharmacological treatment, angiographic findings, and comorbidities.

15.5.2 Hyperlipidemia and Atherosclerosis

Many patients with dyslipidemia cannot achieve optimal cholesterol levels with existing pharmacological therapies [40] (Chap. 28). Therefore, hypercholesterolemia is a promising target for gene therapy. Familial hypercholesterolemia (FH) is an inherited monogenetic disorder caused by low-density lipoprotein (LDL) receptor deficiency. A considerable number of proof-of-principle research studies have been performed in animal models of homozygous FH [41]. The first pilot study of liver-directed gene

therapy in patients with FH demonstrated significant and prolonged reductions in LDL cholesterol [42]. In general, gene therapy approaches for atherosclerosis are progressing in two directions: to decrease cholesterol levels in the blood through liver-directed molecular therapy and to target atherosclerotic lesion directly or atherosclerosis-related vascular complications [43]. Apolipoprotein B100 (ApoB) is the main form of LDL and therefore a major target for gene therapy. Blockade of serum ApoB mediated by short interfering RNAs (siRNA) induced up to a 95 % reduction of liver ApoB mRNA and serum ApoB protein and a significant reduction of serum LDL in a mouse model with humanlike lipid profile [44]. Inhibition of ApoB synthesis with mipomersen (second-generation antisense oligonucleotide) decreased LDL cholesterol concentration by 25 % in 34 patients [45]. Another gene therapy product modulating cholesterol level is PCSK9, which binds to LDL receptors. A phase 1 trial demonstrated that inhibition of PCSK9 synthesis by RNA interference (RNAi) in 32 participants is a potentially effective mechanism to reduce LDL cholesterol [46]. Endothelial nitric oxide synthase (eNOS) gene transfer enhanced the antiatherogenic parameters of the atherosclerotic vessels and can reduce inflammatory cell infiltration and wall lipid deposition. Another way to decrease inflammation in atherogenic processes is targeting NF- κ B which acts as a regulator of inflammatory events in endothelial cells [47].

15.5.3 Primary Systemic Hypertension

Hypertension has adverse effects on cardiovascular function and is a major risk factor for development of aortic dissection, intracerebral hemorrhage, ischemic heart disease, peripheral vascular disease, and renal insufficiency. The most effective antihypertensive drugs are short-term acting, must be taken everyday, and produce significant side effects. Finally, all of these medications decrease symptoms but do not cure the underlying causes; thus, their discontinuance results in the reappearance of high blood pressure (Chaps. 30 and 31). Genetic manipulation for hypertension in theory can induce a permanent effect with precise specificity based on molecular structure and, on a conceptual level, such an approach would be better than pharmacological therapy.

Potential gene strategies for control of hypertension are (i) use of antisense oligonucleotides to inhibit genes involved in elevated blood pressure pathways, like members of the renin-angiotensin system, angiotensin II, and beta-adrenergic receptors, and (ii) overexpression of genes encoding proteins that induce vasodilatation like kallikrein, atrial natriuretic peptide, or endothelial NOS.

15.5.3.1 Overexpression of Vasodilator Genes

DNA construct containing the human eNOS caused a significant reduction of systemic blood pressure for 6 weeks in hypertensive rats [48]. A single injection of the human adrenomedullin gene resulted in a prolonged reduction of blood pressure with a maximal reduction of 41 mmHg 9 days after gene delivery [49]. A maximal

blood pressure reduction of 50 mmHg was observed in rats receiving kallikrein gene delivery, as compared to rats receiving only marker gene [50]. These and other studies provide support for the use of vasodilator gene overexpression to control hypertension.

15.5.3.2 Antisense Knockdown Approach

Downregulation of angiotensinogen mediated by antisense oligonucleotides effectively reduced blood pressure in rats with cold-induced hypertension [51]. A prolonged antihypertensive effect of angiotensin type 1A receptor downregulation via antisense oligonucleotide was shown in a renovascular model of hypertension [52]. Antisense inhibition of β_1 -adrenergic receptor mRNA has advantages over currently used β -blockers in providing a profound and prolonged reduction in blood pressure without affecting heart rate in spontaneously hypertensive rats [53].

Despite these and other positive results, several issues should be resolved before this strategy will be implemented in practice:

- (i) Creation of experimental models with the possibility to reverse established hypertension
- (ii) The development of a vector system that can regulate transgene expression
- (iii) The discovery of an ideal gene target for hypertension
- (iv) The assessment of safety controls for viral gene delivery systems [54]

15.5.4 Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a hemodynamic and pathophysiological condition involving abnormal proliferation of vascular endothelial cells with intimal thickening and muscularization of the distal pulmonary arteries, which leads to an increase in mean pulmonary arterial pressure of more than 25 mmHg at rest. Different congenital and acquired cardiovascular diseases are associated with PAH. Development of obstructive pulmonary vascular remodeling ultimately causes right-side heart failure with a 5-year mortality rate of 50 % [55] (Chap. 45 and 59).

During the past decade, patients with familial PAH were found to have germline heterozygous mutations in bone morphogenetic protein receptor II (BMPR2). Mutations in BMPR2, a member of the transforming growth factor-beta (TGF-beta) receptor superfamily, resulted in cell proliferation and differentiation in the pulmonary tree [56]. Activation of the BMPR2 axis led to the suppression of proliferation and the activation of apoptosis in human pulmonary artery smooth muscle cells [57]. BMPR2 replacement gene therapy appears attractive to correct heredity-caused PAH [58].

Nitric oxide (NO) synthesized by eNOS is a potent vasodilator and is considered to play an important role in the proliferation of pulmonary vascular smooth muscle cells. Overexpression of eNOS-derived NO significantly attenuated muscularization

of small arterioles and therefore inhibited remodeling of the pulmonary vasculature induced by hypoxic pulmonary vasoconstriction [59]. Smooth muscle cell proliferation and migration characterize the pathogenesis of pulmonary hypertension.

Calcitonin gene-related peptide (CGRP) has a high pulmonary vascular activity which is reduced in the case of pulmonary hypertension. Studies have shown CGRP can inhibit pulmonary smooth muscle cell proliferation by binding to CGRP receptors [60]. The lungs of rats transplanted with CGRP-expressing endothelial progenitor cells demonstrated a decrease in both mean pulmonary artery pressure and total pulmonary vascular resistance at 4 weeks. Morphologic examination of pulmonary vasculature also showed that pulmonary vascular remodeling was remarkably inhibited as the vascular medial wall thickness was reduced [61].

In certain etiologies of PAH, the gene therapy approach is required to use short-acting proteins such as prostacyclin synthase (PS) [62]. PS catalyzes the synthesis of prostacyclin and PS deficiency plays a part in the development of PAH. PS has a major role in modifying the pulmonary vascular response to chronic hypoxia and its overexpression protected lungs of transgenic mice from PAH [63].

Inhalation of the vasodilator peptide adrenomedullin can decrease pulmonary vascular resistance in patients with idiopathic PAH [64]. Other genes that have shown therapeutic effects in PAH include vascular endothelial and hepatocyte growth factors which possess angiogenic effects that cause lung regeneration and protect from endothelial injury.

15.5.5 Peripheral Arterial Disease

Peripheral arterial disease is a slowly progressing vascular circulatory disorder caused by the advance of atherosclerosis. Clinical manifestations accompanying poor prognoses include development of ischemic lesions, claudication, intractable rest pain, and critical leg ischemia.

Alternative applications of gene therapy include management of vascular stenosis, restenosis, and postoperative graft failure. Therapeutic angiogenesis improves tissue perfusion through the growth and proliferation of blood vessels after delivery of angiogenic growth factors. A high number of experimental and clinical trials were performed with vascular endothelial growth factors (VEGF), hepatocyte growth factor, fibroblast growth factor, and hypoxia-inducible factor-1 α . Increased resting and maximum flow after intra-arterial administration of VEGF mediated by plasmid was first demonstrated in humans with critical leg ischemia 18 years ago [2]. There have since been about 20 clinical trials with proangiogenic cytokines. The main objectives pursued by therapeutic angiogenesis are:

- (i) Proliferation and migration of endothelial cells followed by formation of new vessels
- (ii) Remodeling of preexisting collaterals
- (iii) Improvement of vessels' vasomotor function
- (iv) Tissue regeneration [65].

The ability of gene-based therapy to alleviate the pathophysiological changes in PAD was demonstrated in diverse animal models and multiple clinical trials. However, for the achievement of positive long-term clinical results, several obstacles must be overcome: transient therapeutic gene expression, off-target effects, selection of patients suitable for clinical trials, and identification of the ideal vectors and delivery routes [36].

15.5.6 Heart Failure

Modern pharmacological therapy for heart failure (HF) requires the use of many drugs at the same time which can lead to complex drug interactions and side effects. Despite the fact that IHD is the most common cause of HF, gene therapy targets for the treatment of these two diseases differ [66].

Gene therapy to rescue the failing myocardium in HF has focused primarily on excitation-contraction coupling and reduction of adverse remodeling regardless of etiology [67]. The main molecular targets are calcium cycling proteins, the β -adrenergic system, homing of stem cells, and apoptosis [68]. Targeting Ca cycling proteins in turn involves *overexpression of SERCA2a* [69–71], *phospholamban inhibition* [72], and *S100A1 overexpression* [73]. β -adrenergic signaling targets include *overexpression of β -AR* [74], *inhibition of GRK2 with β ARKct* [75, 76], and *activation of adenylyl cyclase expression* [77]. The ability to promote the homing of stem cells in ischemic cardiomyopathy was demonstrated with the SDF1/CXCR4 complex [78].

15.5.6.1 SERCA2a

Dysregulation of intra- and extracellular calcium cycling transport which occurs via the sarcoplasmic reticulum (SR) calcium adenosine triphosphatase (Ca^{2+} pump (SERCA2a)) in both the systolic and diastolic phases is one of the major characteristics of HF. SERCA2a downregulation or inhibition causes a prolongation of Ca^{2+} transient and an increase in systolic and diastolic intracellular Ca^{2+} [68, 79]. Improvement of contractility has been demonstrated in a number of experimental and clinical studies. Cardiac overexpression of SERCA2a in HF significantly improved LV function, decreased markers of oxidative stress, decreased both myocyte apoptosis and hypertrophy, and arrested adverse remodeling in large animal ischemic cardiomyopathy [70, 71] (Chap. 4). The first SERCA2a clinical trial in patients with HF began in 2008 [80]. At 6 months follow-up, patients reported improvement and stabilization of clinical symptoms and functional tests [69].

15.5.6.2 AC6

Adenylyl cyclase (AC6) regulates the transition of adenosine triphosphate to cyclic adenosine monophosphate and initiates many cardiac intracellular and extracellular signaling pathways. Diminished AC6 activity is associated with downregulation and desensitization of β -adrenergic receptors in HF [77]. Intracoronary delivery of

adenovirus encoding AC6 halted LV remodeling in different HF models. A clinical trial of AC6 gene transfer for HF is in progress [81].

15.5.6.3 SDF-1/CXCR4

Stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 play an important role in the process of stem cell mobilization to the ischemic cardiac environment and are therefore crucial for myocardial repair. Endocardial delivery of DNA plasmid encoding SDF-1 in 17 patients with ischemic cardiomyopathy (NYHA class III) demonstrated with improvement in 6-min walk distance and quality of life [78].

15.5.6.4 β AR

β -Adrenergic receptor (β AR) signaling system plays a pivotal role in cardiac function and has emerged as an attractive therapeutic molecular target in HF [82]. The carboxyl terminus of the β -adrenergic receptor kinase (β ARKct), a competitive inhibitor of G protein-coupled receptor kinases (GRKs), has the potential to resolve β AR signaling abnormalities related to HF (Chap. 5). β ARKct delivery enhanced cardiac contractility and increased adrenergic reserve in a large animal model [76]. The same trend was observed in different HF models [83, 84]. With an increasing basic understanding of cardiac pathology, the establishment of more efficient techniques of gene transfer, and the discovery of new molecular pathways, gene delivery will undoubtedly become an essential complement to conventional therapy of heart failure.

15.5.7 Cardiac Arrhythmias

Many cardiovascular diseases are associated with impairments to the heart's electrophysiological function. The current available therapeutic options are far from perfect. Indeed, ventricular arrhythmias were detected in 43 % of human sudden death cases [31] and several clinical trials have associated antiarrhythmic pharmacotherapy with increased mortality [85] (Chap. 52). The development of new gene transfer methods with percutaneous cardiac catheterization procedures has placed arrhythmias within reach of gene therapy. This strategy targeting arrhythmias includes heart rate control and biological pacemaker function, repolarization and prolonged QT interval, and modulation of cardiac conduction [86, 87].

Atrial fibrillation (AF) is the most common chronic arrhythmia associated with an adverse prognosis. It is estimated that 2.2 million Americans have intermittent or sustained AF. AF typically appears secondary to other heart diseases such as hypertension, valvular disease, or ischemic heart disease (Chap. 50). Gene-mediated rate control was demonstrated in a physiologically relevant model of persistent AF. After 3 weeks of atrial fibrillation, swine received atrioventricular nodal gene transfer with adenovirus encoding the cGi gene. cGi caused a sustained 15–25 % decrease in heart rate and resulted in reversal of the clinical symptoms [88].

One of the main manifestations of failing myocytes is delayed terminal repolarization with QT interval prolongation. Adenoviral gene delivery of KCNE3, a regulatory component of the delayed rectifier potassium channel, accelerated cardiac repolarization and corrected the QT interval [89]. Targeted overexpression of connexin-43 in the healed infarct border zone improved conduction velocity and reduced ventricular tachycardia susceptibility [90]. In a postinfarcted large animal model with reproducibly inducible ventricular tachycardia, the effects of KCNH2-G628S gene transfer on the arrhythmia were evaluated. One week after gene delivery, all transgenic animals had complete elimination of ventricular arrhythmia [91]. Continuing research in this area may result in successful clinical application of gene therapy for the treatment or prevention of arrhythmias.

15.5.8 Gene Therapy Breakthrough and Challenging Opportunities

The concept of gene therapy was introduced in the 1970s after the development of recombinant DNA technology. Despite initial great expectations, the field was slowed down by the failure of initial clinical trials, reports of well-known complications, and both safety and ethical concerns. These early setbacks resulted in widespread skepticism, which to a certain degree persists to this day. However, recent successes of gene therapy undoubtedly inspire molecular biologists and geneticists, including treatment of Leber's congenital amaurosis after subretinal delivery of recombinant adeno-associated viral vector carrying RPE65 gene [92], progress in X-linked adrenoleukodystrophy patients treated with genetically corrected ex vivo CD34 cells [93], and achievement of remission in chemotherapy-refractory B-cell lymphoma after injection of genetically modified T cells [94]. Since 2008, several very promising clinical trials have begun in ischemic heart disease and heart failure including targeting calcium cycling proteins (sarcoplasmic reticulum calcium adenosine triphosphatase overexpression, phase I and phase II/III), effector molecules for β -adrenoreceptors (adenylyl cyclase overexpression, phase I), and endocardial delivery of DNA plasmid encoding stromal cell-derived factor (phase I). These clinical trials based on molecular biology demonstrate a new direction for cardiovascular gene therapy which, unlike surgical approaches, can improve heart and blood vessel function by correcting the pathophysiological disease mechanism on the cellular level. And unlike pharmacological approaches, gene therapy can achieve long-term benefit in chronic cardiovascular diseases from as few as a single treatment.

15.6 Concluding Remarks

Gene therapy is a very promising and rapidly growing part of contemporary medicine, and it is very important to correctly implement it in the treatment of cardiovascular disease. Much effort has been devoted to overcoming safety and ethical

concerns. Nevertheless, questions still remain about the potential for mutagenesis and malignant transformation when certain viral vectors integrate into the host genome, vector toxicity, and the potential for an immune response. Other issues currently being investigated include restriction of collateral organ biodistribution, targeting of specific cells, and relatively low transduction efficiency. Clinically reliable delivery techniques also require improvement to create a minimally invasive closed recirculatory system. Optimization of these issues would allow for gene therapy to become an accepted treatment for cardiovascular disease.

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