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Gene Therapy Approaches to Cardiovascular Disease

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Summary

The potential of enhanced cardiovascular function via gene therapy has aroused extensive interest. Both viral and nonviral vectors have shown promise in the realm of cardiovascular gene therapy. Modification of vectors or addition of further transgenes to the expression cassette has permitted targeted and regulated gene expression. The many potential targets of cardiovascular gene therapy can be considered under the following headings: vascular, congenital heart disease, and myocardial. Cardiac gene delivery may be to either the endothelium of either native coronary vessels or coronary artery bypass grafts, or to the myocardium. Myocardial gene delivery is possible either via direct myocardial injection or via the coronary vasculature.

However, alteration of any cardiac cellular signaling pathway may have cardiotoxic effects. Thus, any genes that appear to cause enhanced cardiac function, must undergo extensive toxicity studies in animals before similar experiments are performed in human subjects. The techniques described may be utilized in the future to deliver various genes targeted to combat many different disease processes, in different animal models, and ultimately to provide feasible gene therapy approaches to human cardiovascular disease.

Key Words: Gene transfer; viral vector; nonviral vector; transcriptional targeting; translational targeting; regulatable transgene expression; intracoronary; coronary catheterization; cardiac surgery; cardiopulmonary bypass; cardiac-selective; adrenergic receptor; vascular; endothelial; myocardium.

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1. Introduction

The potential of enhanced cardiovascular function via gene therapy approaches has aroused extensive interest ***(1–4)***, and in this chapter we review some of the major areas. First, the role of both viral and nonviral vectors in the realm of cardiovascular gene therapy is considered. Second, the methods of targeted and regulated gene expression by modification of the vector or addition of further transgenes to the expression cassette are explored. Third, an overview is given of some of the existing animal models whereby vector delivery can be achieved. Finally, we discuss the potential targets of cardiovascular gene therapy.

2. Vectors Available for Cardiac Gene Therapy

Many vectors have been advocated for gene transfer. These may be classified as either viral or nonviral ***(5,6)***.

2.1. Viral Vectors

The viral genome contains the essential viral genes, genes required for replication and structural products, and a packaging domain. These regions are surrounded by regulatory sequences. After cell entry, replication and associated activity of the early genes occurs. This is followed by activity of the late genes and packaging of new viral particles prior to release of these new virions ***(7)***.

Generation of a viral vector system involves the creation of vector DNA as well as helper DNA (**Fig. 1**). Vector DNA contains the regulatory sequences and a packaging domain, but a therapeutic expression cassette replaces the essential viral genes. This vector DNA is incapable of replication without the assistance of the helper DNA. The helper DNA contains the essential viral genes surrounded by regulatory sequences placed in a heterologous or unrelated DNA context. This may be a plasmid, a helper virus, or the host chromosomal DNA of the packaging cell. The helper DNA does not contain a packaging domain, and thus, in itself, is incapable of producing new virions ***(6)***.

2.1.1. Adenovirus

Adenoviruses have a 36-kb double-stranded DNA genome that contains five early transcription units (E1A, E1B, E2, E3, and E4), two delayed early units (IX and IVa2), and one late unit (major late). This late unit is processed to generate five families of late mRNAs (L1–L5). Following cell entry, proteins in the viral particle are efficient at endosomal lysis. This allows the virus to escape, and subsequently the genome enters the nucleus. The E1 gene acts as a master transcriptional regulator originating the process of viral gene expression leading to viral replication. E1 in combination with the E2 and E4 genes is required for viral genome replication. E3 genes are dispensible for the viral life

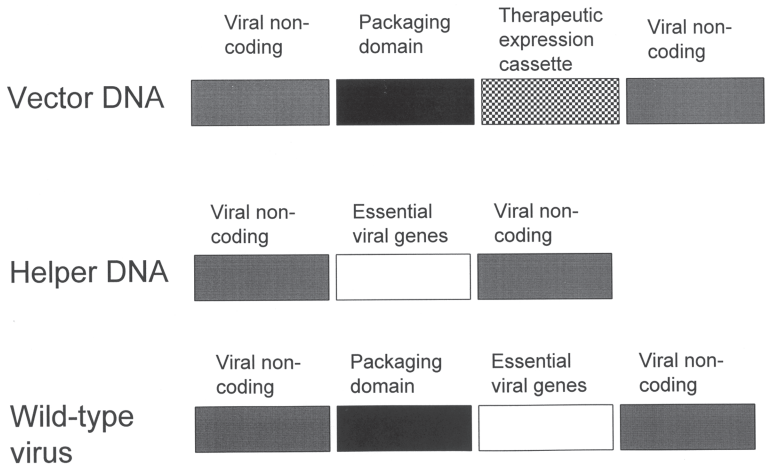

Fig. 1. Engineering virus into vector.

cycle. "Late" genes encode viral structural protein genes that are transcribed, allowing for encapsidation of the newly replicated genomes ***(8)***.

First-generation adenoviral vectors have an E1 deletion. The E1 deleted viral genome containing the transgene is added to a cell line that contains a stable E1a expression cassette. This allows the added DNA to replicate and be packaged into E1 deleted vectors, which, in theory, are not capable of replication. However, even in the absence of E1 gene products, transcription occurs at a low rate, inducing an early inflammatory cytokine response. This is followed by an antigen-dependent immune response that includes the cell-mediated destruction of transduced cells ***(9)***. The E3 gene may play a role in immune surveillance in infected hosts and deletion of it may offer protection against some of the immune-mediated responses directed against the vector or vector-transduced cells.

There are more than 50 different human adenoviral serotypes ***(8)***. The possibility of using adenovirus as a vector was demonstrated following systemic intravenous delivery in neonatal mice ***(10)***. This resulted in widespread β-galactosidase (β-gal) expression that persisted for almost 1 yr ***(11)***. Adenoviral vectors can efficiently infect nondividing cells. The DNA remains episomal, rather than being incorporated into the host cell chromosome, although the period of persistence of the episomal genes is unknown. Current vectors are primarily derived from types 2 and 5, the most common serotypes to which adults have been exposed. It may be possible to use other serotypes or nonhuman adenovirus to avoid potential problems related to preexisting immunity.

Secondary vector delivery using a different serotyped capsid has also been demonstrated in animal models ***(12)***. Adenoviral vectors have many favorable characteristics suggesting their utilization as vectors for gene transfer. They can infect a wide range of target cells including nondividing cells, and they can be produced in high titers with relative ease. Despite initial concerns over the possibility of germline gene insertion, it appears that adenoviruses cannot infect oocytes and so the risk of female germline transduction is low ***(13)***. However, limitations include the generation of an inflammatory and immune response, which may be related to the relatively short duration of gene expression ***(14–18)***. To avoid the immune response to the vector, it may be possible to use newer, improved adenoviral vectors. An adenoviral vector with deletion of E2b, in addition to E1 and E3, has diminished late adenoviral gene expression compared with the conventional vector ***(19)***. This vector can also be produced with relative ease in large quantities and so may be suitable for large animal studies.

Recent interest has focused on high-capacity, "gutted" or "gutless" adenoviral vectors ***(20,21)***. These vectors have two main advantages over earlier generation adenoviral vectors. First, all viral coding sequences are deleted from the vector genome, so that viral proteins are not expressed from the vector, thus reducing its toxicity. Second, with the absence of viral coding sequences, the capacity for incorporation of heterologous DNA is increased to 36 kb, allowing the simultaneous expression of several genes, large cDNAs, and regulatory elements. However, transgene expression with "gutted" adenoviral vectors has been shown to be lower than with earlier generation adenoviral vectors, although this was corrected by addition of the E4 region of the adenovirus genome ***(20)***. It has been established that additional stuffer DNA has to be included in a "gutted" adenoviral vector genome if the gene or the expression cassette that is incorporated is less than 27 kb in size. This stuffer DNA may contain nuclear matrix attachment regions which can also influence gene expression ***(21)***.

2.1.2. Adeno-Associated Virus

Adeno-associated virus (AAV) is a human parvovirus that is not able to replicate unless a helper virus, such as adenovirus or herpesvirus, is present in the same cell. There are six known human serotypes but AAV-2 has been the major focus of attention. AAV has not been linked to human disease and can infect a wide range of target cells, establishing latent infection by integration into the cell genome. The viral genome consists of two genes, the *rep* gene required for viral genome replication and the *cap* gene, which encodes structural proteins. These two genes are flanked by viral inverted terminal repeats (ITRs). AAV vectors can be produced by adding separate plasmids containing the ITRs flanking the therapeutic gene cassette, the *rep* and *cap* genes, and the

addition of a helper adenovirus or another plasmid containing the essential adenoviral helper genes ***(6)***. AAV efficiently transduces both dividing and nondividing cells, through both episomal transgene expression and by random chromosomal integration. It has given stable β-gal expression 8 wk after vector delivery by intramuscular cardiac injection to mice and 6 mo expression after intracoronary delivery to the pig circumflex artery, with no evidence of cardiac inflammation or myocardial necrosis ***(22,23)***. This may be owing to the absence of viral coding sequences from the AAV vector genome. However, the maximum transgene size is approx 5 kb, and production is currently very labor-intensive; this may improve with recent advances. Furthermore, it is not usually produced in as high a titer as recombinant adenovirus ***(24)***. In vivo expression has a delayed onset with minimal expression until 1 mo ***(25)***. This may be related to a requirement for generation of double-stranded (ds) DNA genomes by either vector single-stranded (ss) DNA annealing, or second-strand synthesis followed by vector genome linking. Moreover, vector genome linking has allowed effective doubling of the limited coding capacity by splitting an expression cassette into two vectors and administering them simultaneously ***(6)***.

2.1.3. Retrovirus

Retroviruses encode RNA-dependent DNA polymerase, reverse transcriptase, which converts the viral ss RNA genome to ds DNA, which is then inserted into the host chromosome. For this integration to occur, the infected cell must undergo mitosis shortly after infection, thus limiting the delivery of DNA to replicating cells. Retroviral genomes have two long terminal repeat (LTR) sequences at their ends. These frame the *gag*, *pol*, and *env* genes encoding the structural proteins, nucleic acid polymerases/integrases, and surface glycoprotein, respectively. A transgene of up to 8 kb can be inserted and expressed in place of the viral genes, which are expressed by heterologous transcriptional signals from two separate constructs lacking most viral *cis*-acting sequences and stably incorporated in packaging cell lines. This split construct design improves vector safety by increasing the number of recombination events required to reconstruct a replication-competent genome.

Lentiviruses have a more complex genome, which encodes two additional regulatory genes, *tat* and *rev*, essential for expression of the genome, and a variable set of accessory genes. Unlike retroviruses, they rely on active transport of the preintegration complex into the nucleus of the target cell, which allows integration into the host genome in the absence of replication. In addition, they do not trigger an inflammatory response. Safety concerns remain regarding the use of these members of the human immunodeficiency virus (HIV) family, including potential mutation into its pathogenic phenotype. New generations of vectors that contain only the essential packaging genes may

increase vector biosafety. Moreover, self-inactivating transfer vectors contain a deletion in the downstream LTR that, when transduced into target cells, results in the transcriptional inactivation of the upstream LTR and reduces the risk of vector recombination *(6,26)*. A lentivirus-based vector system has been developed that can transduce nondividing cardiac myocytes and integrate the transgene into the genome of target cells *(27)*. However, generation of lentiviral vectors is also labor-intensive. Moreover, the issue of attenuation of gene expression owing to vector silencing remains unresolved. It has been suggested that multiple integration events may need to be achieved for long-term expression, since probably only a proportion of the integrated vectors remain transcriptionally active *(26)*. Restraints are placed on the viral genome by the obligatory RNA step in the retroviral life cycle such that the transgene expression cassette must be of limited size and without introns or internal polyadenylation signals *(6)*.

A summary of some characteristics of these viral vectors is shown in **Table 1**.

2.2. Nonviral Vectors

Cationic liposomes rely on receptor-mediated endocytosis or fusion of cell membranes. Negatively charged DNA is contained within a positively charged lipid vesicle. Plasmid DNA is released in the cytoplasm, but only a small proportion of it enters the nucleus. There it remains separate from the host genome and is associated with transient transgene expression. Liposomes have a favorable safety profile. They contain no viral sequences, and, unlike viral vectors, they have no cDNA size constraints in vector construction. Efficiency of transfection is enhanced in proliferating cells, although cell division is not required *(5,27)*. The role of nonviral vectors has been thought to be limited owing to their markedly reduced efficiency of myocardial gene transfer *(5,28–30).* However, in a functional heterotopic rabbit heart transplant model, despite lower efficiency of liposome-mediated gene transfer, the efficacy of liposome-mediated interleukin-10 gene transfer was much higher than that mediated by adenovirus. Significant negative inotropic and arrhythmogenic adverse effects on transplanted hearts were observed owing to viral cytotoxic and immune responses, which attenuated the therapeutic efficacy of the first-generation adenovirus-mediated gene therapy *(31)*. A carrier system incorporating low-density lipoprotein (LDL), lipids, and plasmid DNA has demonstrated enhanced myocardial transfection, which may be related to LDL receptor-mediated endocytosis *(32)*.

Plasmid DNA and RNA can be directly injected into skeletal and cardiac muscle. Antisense oligonucleotides are short DNA sequences complementary to the RNA message of interest. These are chemically modified to resist nuclease degradation. After direct injection, much of it is degraded in the intra-

Table 1
Properties of Different Viral Vectors for Cardiovascular Gene Therapy

	Adenovirus	Adeno-associated virus (AAV)	Retrovirus
Ease of production	Produced in high titers with ease	Moderately high titers but labor-intensive	Low titers
Onset of expression	Early	Delayed	Delayed
Duration of expression	Short term	Prolonged	Prolonged but vector silencing may occur
Infect nondividing cells	Yes	Yes	No, except lentivirus
Inflammatory/immune response	Marked, but reduced with "gutted" adenovirus	Relatively nonimmunogenic	Relatively nonimmunogenic
Integration into host genome	No, episomal	Both episomal and chromosomal insertion	Chromosomal insertion, potential for mutagenesis

cellular lysosomal compartment. Only a fraction, therefore, escapes intracellular or extracellular degradation. Transfection efficiency is lower than with liposome complexes and adenoviral vectors ***(28,29)***. The nucleotide sequence hybridizes to target RNA, which prevents translation of RNA. However, antisense oligonucleotides bind to other mRNAs showing partial similarity to the complementary target RNA, and even to various proteins, which can cause altered functional characteristics ***(5,30)***.

3. Regulation of Gene Expression

Expression levels of an introduced gene depend mostly on the transduction efficiency of the vector and on the strength of the transcriptional regulatory elements. Typically, strong and ubiquitously active viral promoters such as human cytomegalovirus (CMV) have been used to drive transgene expression ***(33)***. A complication of protracted, unrestricted gene expression, which could be offered by many of the newer viral vectors, is the potential for deleterious side effects ***(34)***. Thus, it has been suggested that regulatable promoters and vectors are required ***(35)***. Several systems for controlling exogenous transgene expression have been used including steroid hormones such as glucocorticoids ***(36)***. A limitation of using steroids is the interference of endogenous gene

expression. The tetracycline-controllable expression system enables tight on/off regulation, high inducibility, fast response times, no pleitropic effect owing to the use of the tetracycline operon derived from bacteria, and a well-characterized inducer, namely, tetracycline or doxycycline ***(37)***. Two types of system, tet-off and tet-on, have been used to regulate transgene expression. In the tet-off system, the tetracycline-responsive transcriptional activator (tTA) induces the transcription of a gene containing the tet-responsive element, and transcription is turned off in the presence of tetracycline. In the tet-on system, the reverse tetracycline-responsive transcriptional activator (rtTA), binds to the tet-responsive element and turns on the transcription in the presence of tetracycline. In an adenovirus-mediated tetracycline-controllable expression system, the tet-off system was demonstrated to be functionally superior, in terms of tightly regulatable transgene expression ***(38)***.

The acetylation state of histones is correlated with gene expression, in that deacetylated histones are associated with repressed gene expression. A single vector system that contained a chimeric tetracycline repressor, which interacts with histone deacetylases, and a tetracycline-sensitive promoter was created. In vitro experiments showed tight control of gene expression in a doxycycline dose- and time-dependent fashion ***(39)***. Tetracycline regulation has been combined with glucocorticoid regulation in chimeric promoters ***(40)***. Tetracycline-dependent lentiviral vectors have also been developed, resulting in efficient regulation of transgene expression ***(41)***. A hypoxia-regulatory element, which is activated by transactivating hypoxia-inducible factor (HIF)-1 in response to a reduction in oxygen has been combined with a cardiac-specific promoter in an AAV vector. This "vigilant vector" has been tested to give proof of principle that therapeutic genes could be switched on in the heart during times of ischemic stress ***(42)***.

4. Transductional and Transcriptional Targeting

Gene expression following systemic adenoviral vector delivery is affected by the natural tropism of the virus for the coxsackie/adenoviral receptor (CAR). CAR is expressed to high levels in the liver, which consequently has a high capacity for nonspecific vector uptake. Transductional targeting alters the natural infection pathway and can be performed by pseudotyping, whereby modulation of the viral envelope allows manipulation of viral vector cell targeting specificity. This has been performed in a range of vectors including adenovirus, AAV, and lentivirus ***(43)***. It has been possible to perform transductional targeting to, for example, the pulmonary endothelium ***(44)***.

Transcriptional targeting via tissue-specific promoters has also been investigated. A suitable cardiac-specific promoter is the myosin light chain ***(42,45)***. An additional benefit of tissue-specific promoters is the reduction of gene

expression in antigen-presenting cells, thus reducing the host immune response ***(46)***. However, a limiting factor in the use of tissue-specific promoters is the lower level of expression compared with their viral counterparts, such as the CMV promoter. This can be overcome by constructing synthetic promoter libraries or by exploiting endogenous genomic sequences that enhance tissue-specific expression ***(47)***. A further problem with such tissue-specific promoters is that the selectivity of some promoters is altered or lost when they are placed in the context of an adenoviral genome. It has been demonstrated that enhancer elements or transcription start sites within the adenoviral sequences surrounding the transgene expression cassette interact with the promoter elements, which can activate transcription in nontarget cells ***(48,49)***.

5. Animal Models

Cardiac gene delivery can be performed with targeting of genes to the endothelium of either native coronary vessels or coronary artery bypass grafts, or to the myocardium. Myocardial gene delivery can be performed either via direct myocardial injection or via the coronary vasculature. Direct myocardial injection requires multiple injections causing high levels of gene expression within a 1.5-cm radius of the site of injection ***(50–52)***.

Intracoronary delivery of vector in a Langendorff perfused heart model has shown that prolonged contact time with the virus improves gene transfer ***(53)***. Adenoviral-mediated gene transfer has been achieved during transplantation of rat and rabbit hearts, but this technique is limited to viral exposure of the donor heart prior to transplantation ***(54–56)***. Intracoronary delivery in rats and rabbits has been performed during thoracotomy, with the aorta cross-clamped and the virus introduced by injection into the left ventricle (LV) cavity ***(57–60)***. Owing to the occluded aorta, LV ejection forces the virus down the coronary arteries. However, the acute pressure overload of the LV limits the period of cross-clamping to a matter of seconds. A more clinically applicable method of gene delivery is direct catheterization of the right or left coronary artery. Here too, exposure of the heart to the virus is limited. Present systems require high infusion pressure to inject the virus, which could result in endothelial or myocardial injury ***(61,62)***. Following these methods of gene delivery, the adenovirus was also distributed systemically, and noncardiac tissues have shown evidence of transgene expression ***(57,58,63)***. We have therefore developed a cardiac-selective method of vector delivery in which the vector is delivered to the cardioplegic arrested heart following application of an aortic cross-clamp during standard cardiopulmonary bypass (CPB) (***64,65***; *see* Chapter 4).

Continuous circulation of an adenoviral vector solution through the heart has been suggested as another potential method of gene delivery during CPB, although this requires the use of two separate circuits and oxygenators, unlike

the standard approach *(66)*. Gene transfer has been documented using retrograde delivery of vector through the coronary sinus in a porcine beating heart model *(67)*. This is another method that could also be utilized for vector delivery during cardiac surgery. Additional work in the Langendorff perfused rabbit heart suggested that agents that increase endothelial permeability can greatly enhance the efficiency of gene transfer *(68)*. Further experiments have demonstrated that vascular endothelial growth factor (VEGF), which is known to produce a transient increase in microvascular permeability within the coronary circulation, can also enhance escape of virus particles from the vasculature *(69,70)*. Increased myocardial transcapillary gradient and high-pressure infusion of adenoviral vectors are some of the physical methods that have been demonstrated to optimize myocardial gene transfer *(69,71)*. Utilization of these principles has been used to deliver a deficient structural protein, δ-sarcoglycan, to cardiomyopathic hamsters, resulting in a slowing of the progression of LV dysfunction compared with controls. The method of vector delivery involved deep hypothermia to 18–25°C, followed by the delivery of cold crystalloid cardioplegia containing histamine. This allowed homogenous transgene expression in more than 75% of LV myocytes *(72)*.

Of particular pertinence in this setting is the fact that cardioplegia has detrimental effects on endothelial structure and function *(73,74)*. Contact with cardioplegia and the associated relative ischemia may increase endothelial permeability, thus overcoming one of the barriers to adenoviral infection of cardiac myocytes. It is interesting to note that in a transplant heart model adenovirus-mediated gene transfer was enhanced when the vector had a prolonged dwell time during warm ischemia *(75)*. Furthermore, if delivery of a gene that could enhance postoperative cardiac function was possible during CPB, as we have shown with adenoviral-mediated gene transfer of the human β_2-adrenergic receptor (β_2AR), this might be especially desirable toward the end of a prolonged cross-clamp period when impaired cardiac function may be more likely *(76–76b)*.

6. Targets for Cardiac Gene Transfer

6.1. Vascular

A range of targets for vascular gene therapy exists *(5)*. Therapeutic angiogenesis in ischemic myocardium has been attempted in canine and porcine models using VEGF *(51,77)*. In a pacing model of congestive heart failure, adenovirus-mediated VEGF gene transfer improved cardiac function *(78)*. Moreover, a preliminary placebo-controlled, double-blind trial of naked plasmid-mediated VEGF gene transfer in patients with chronic myocardial ischemia demonstrated a statistically significant reduction in anginal class and

a trend towards improved exercise duration ***(79)***. Fibroblast growth factor (FGF) also stimulates many cell types including endothelial cells ***(80)***.

Restenosis following coronary angioplasty is a significant clinical problem. Gene transfer of an inhibitor of β-adrenergic receptor kinase (βARKct) can alter vascular smooth muscle proliferation via G protein-coupled signal transduction and so reduce the severity of restenosis ***(81)***. Following peripheral arterial grafting and coronary artery bypass grafting (CABG), venous graft occlusion is linked to neointimal hyperplasia ***(82)***. Ex vivo gene transfer of EGF decoy oligonucleotides to saphenous vein grafts has been performed with encouraging results ***(83)***. Similar treatment of venous grafts during CABG could be performed ***(83a)***. Alternatively, the present model of gene transfer could be utilized during CABG, thus allowing treatment of disease in tissues downstream to the location of the graft, via overexpression of a secreted therapeutic protein from the transduced graft ***(84)***.

The etiology of atherosclerosis is multifactorial, and thus many potential targets exist. It may be possible to have cardiac-selective delivery of genes encoding compounds, such as the LDL, very low-density lipoproteins, high-density lipoproteins, or apolipoprotein E, which may alter the progress of atherosclerosis ***(24,85)***.

6.2. Congenital Heart Disease

In many congenital cardiac disorders the development of cardiac chambers is abnormal. For example, the arterial switch operation performed for transposition of the great arteries requires that the morphological LV be able to take on the workload of the systemic ventricle postoperatively ***(86)***. The βAR system is known to influence myocardial remodeling ***(87)***. Delivery of such genes during congenital cardiac surgery may permit "genetic remodeling" and so allow surgery to be performed in infants with a suboptimally prepared LV.

6.3. Myocardial Targets

In vitro gene transfer of the human β_2AR or βARKct to cardiomyocytes isolated from various animal models has confirmed enhanced contraction at the cellular level ***(58–60,88)***. Another protein whose expression is altered in heart failure is SERCA2a, which is involved in mobilization of intracellular calcium stores ***(89)***. Improved survival and cardiac metabolism after adenoviral-mediated gene transfer of SERCA2a has been shown in a rat model of heart failure ***(90)***. Infection of cardiomyocytes, isolated from failing human LV at the time of transplantation, with adenoviral vector containing the SERCA2a gene has documented improved myocyte contraction compared with infection with empty vector ***(91)***. Similar experiments have also confirmed that adenovi-

ral gene transfer of antisense phospholamban likewise improves contractile function in failing human cardiomyocytes ***(92)***. Proof of benefit of β_2AR and βARKct gene transfer in human tissue has been obtained ***(92a)***. Although such in vitro studies have limitations, it will be of interest to document the effects of transfer of the β_2AR and βARKct transgenes in these models prior to undertaking in vivo human clinical studies of gene transfer.

Cardiac-specific overexpression of β_2AR and βARKct is associated with enhanced LV function in transgenic murine models. Moreover, cross-breeding transgenic heart failure models with a βARKct-overexpressing strain has confirmed rescue of the failure phenotype ***(93–95)***. These beneficial effects have been confirmed in an adenoviral-mediated gene transfer model to rabbits with chronic heart failure subsequent to myocardial infarction ***(60)***. Evidence suggests that βARK1 inhibition is beneficial in many models of heart failure, perhaps by dampening sympathetic overdrive ***(96)***. Moreover, the combination of βARKct expression and chronic βAR antagonism by metoprolol has been shown to increase survival and improve cardiac function in the calsequestrin-overexpressing mouse model of heart failure ***(95)***. In a neonatal porcine model we have confirmed that cardioplegic arrest and CPB induces downregulation of the βAR system, with associated impairment of LV function, which can, however, be ameliorated with β_2AR gene transfer ***(76b)***. A Langendorff perfused rabbit heart model demonstrated elevation of βARK1 following cardioplegic arrest. Interestingly, pretreatment with an adenoviral vector encoding the βARKct transgene restored LV function to normal ***(97)***. It would be of further interest to develop an adult porcine model of heart failure and assess whether gene transfer of β_2AR or βARKct has beneficial effects. A suitable model may be rapid pacing, which has already been documented to induce heart failure in pigs ***(78,98)***.

β_2AR gene transfer is associated with acute enhancement of cardiac function in a rabbit heterotopic heart transplant model ***(99)***. This raises the possibility of using genetic manipulation of β_2AR signaling as an adjunct to mechanical assist devices to facilitate functional myocardial recovery. Polymorphisms of the human β_2AR exist ***(100–102)***. In addition, a reengineered β_2AR that responds to synthetic agonists has been described ***(103)***. Such targets for gene transfer may allow treatment to be tailored for individuals with particular genetic abnormalities. Alternatively, genes could be overexpressed, but manipulation of the desired effect could be achieved by administration of synthetic pharmacological agents.

A novel cell signaling mechanism has been induced in cardiomyocytes in which a vector encoding the V2 vasopressin receptor was delivered to the heart ***(104)***. Cardiomyocytes do not normally express this receptor, but it did couple

satisfactorily to the cellular second messenger systems, resulting in enhanced LV function.

Other targets for myocardial gene transfer abound. Myocardial apoptosis involves complex regulated cell suicide machinery, in which two main signaling pathways lead to activation of the caspase family of cysteine proteases. These pathways involve, first, death receptor signaling, via substances such as tumor necrosis factor or Fas, and second, release of cytochrome c from mitochondria and subsequent *trans*-activation of procaspase 9 by apoptosis protease-activating factor. In vitro studies showed that caspase 3 induced sarcomeric disruption and reduced myocyte contractility, whereas adenovirus-mediated gene delivery of a caspase 3 inhibitor enhanced LV function in a rapid pacing model of cardiac failure ***(105)***.

Ischemia and oxidative stress are major mechanisms of tissue injury. According to the duration and severity, myocardial ischemia and reperfusion may lead to cell injury and death. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion to oxygen and hydrogen peroxide. Ischemic preconditioning is associated with an increase in the activity of manganese SOD, an isoform found in the mitochondrial matrix. In a rat model of ischemia–reperfusion, adenoviral-mediated transfer of manganese SOD reduced infarct size ***(106)***. The catalytic byproducts of hemoxygenase (HO-1) exert wide-ranging antioxidant and cytoprotective effects. AAV-mediated transfer of the HO-1 gene before myocardial injury conferred long-term myocardial protection from ischemia-reperfusion injury, which may be beneficial as preventive therapy for patients with or at risk of developing coronary ischemic events ***(107)***.

7. Perspectives

An exciting prospect for translating much work from animal models to human studies has been opened up by the recent demonstration of quantitative imaging of transgene expression in living animals ***(108)***. The development of micro-positron emission tomography has allowed improved resolution to approx 2 mm^3. Such technology has been utilized to correlate expression of therapeutic and reporter genes in a time-dependent fashion using inducible promoters ***(109)***. However, it must be remembered that alteration of any cardiac cellular signaling pathway may ultimately turn out to have cardiotoxic effects, including arrhythmias. Thus, any genes that appear to cause enhanced cardiac function must undergo extensive toxicity studies in animals prior to similar experiments being performed in human subjects ***(1,2,34,110)***. Although many issues still remain unresolved, the techniques described in this chapter may be utilized in the future to deliver various genes, targeted to combat many different disease processes, in different animal models, and ultimately to provide feasible gene therapy approaches to human cardiovascular disease.

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