Gene therapy in the cardiovascular system: an update

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Abstract

This update reviews the remarkable progression in several cardiovascular gene transfer domains. The first chemical gene therapy protocols to stimulate angiogenesis in ischemic myocardium are discussed and both the great expectations as well as remaining hurdles are highlighted. In experimental models of restenosis and heart failure gene therapy shows promising results. Important questions regarding vector-related limitations and suboptimal in vivo delivery systems will require expeditious attention for gene therapy to become a more widely applicable option in cardiovascular diseases. © 1999 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Two years ago a focus on cardiovascular gene therapy was published in this journal [1], in which several reviews were presented on different fields of cardiovascular medicine, where gene therapy was emerging as a novel potential treatment modality. While some domains, including therapeutic angiogenesis, have progressed at a remarkable pace, other areas are still in the phase of in vitro and small animal research. On the other hand, the powerful gene therapy technology has contributed significantly to a better understanding of cardiovascular biology. Yet many hurdles remain before gene therapy will make a noticeable impact on the treatment of cardiovascular disease: vector-related inflammation, limited expression span, suboptimal delivery systems, and gene expression levels are among the major concerns. Moreover, treatment strategies are often based on mechanisms identified in small animal models, the relevance of which for patient care needs careful consideration [2].

Cancer patients for whom conventional treatment failed, might be more eager to try novel, and still incompletely validated treatments, while many patients with cardiovascular diseases and their doctors hesitate to consider gene therapy. From this view point, patients suffering from cardiac diseases with few or no therapeutic options, including cardiomyopathies and end-stage heart failure, might be better candidates for gene-based treatments in the near future. Also, in some areas where gene therapy appeared as a promising alternative for failing pharmacological approaches, novel strategies are emerging. For instance, the introduction of brachytherapy in the prevention and treatment of restenosis [3] and the advances in percutaneous revascularization procedures might bear on possible applications of gene therapy in its current state.

This update is not intended as a comprehensive overview of current research in cardiovascular gene therapy, but rather as a concise update on new developments in areas that were highlighted in a former focused issue of \textit{Cardiovascular Research} in 1997 [1] and with special emphasis on recent developments in gene therapy for angiogenesis and vasculoproliferative diseases.

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2. Angiogenesis

The therapeutic implications of angiogenic growth have been clearly identified in recent years in different animal models [4,5], and have led to the initiation of clinical trials in patients with coronary and peripheral vascular insufficiency. Intramuscular injection of naked plasmid DNA encoding the 165 amino acid isoform of human vascular endothelial growth factor (VEGF) induced angiogenesis and decreased ischemia in patients with ischemic ulcers due to peripheral vascular disease [6]. In a landmark study, patients with refractory stable angina who were not amenable to classical revascularization received direct intramyocardial injections of VEGF plasmid via a mini-thoracotomy [7]. Patients had reduced clinical symptoms of angina and objective evidence of reduced ischemia as documented by dobutamine single photon emission computed tomography (SPECT) imaging.

However, the results of these studies have to be considered cautiously because of the absence of a control group. Furthermore, inflammation caused by the vector or even the needle injections might have contributed to formation of new capillaries. The need for a control group was also illustrated by the VIVA trial [8]. In this trial, patients with ischemic heart disease received placebo or intracoronary recombinant VEGF protein followed by an IV infusion. Both in the treated and the placebo group the patients’ clinical status and treadmill tests improved, but the absence of a positive effect led to the premature termination of the study. Catheter-based interventional techniques will undoubtedly provide an easier way to achieve myocardial delivery and will facilitate inclusion of a control group. In this respect, successful gene transfer was obtained using a transmyocardial injection catheter [9]. Pericardial delivery using a transatrial [10] or transmyocardial [11] approach has also been considered for diffusible angiogenic gene products, with the pericardial space serving as a sustained-release reservoir. An alternative approach could be to use the percutaneous PerDUCER device [12], allowing for safe access to the pericardial space via a minimal subxiphoid incision.

To date, the majority of gene-based experiments have been conducted in small animal models, predominantly in the murine ischemic hindlimb model. Decreased angiogenesis in diabetic mice could be overcome by intramuscular injection with adenovirus encoding VEGF [13]. New angiogenic growth factors are also being investigated, including hepatic growth factor (HGF) [14], leptin [15] and thrombopoietin [16]. Of interest, local overexpression of a hypoxia-inducible transcription factor has been shown to augment blood flow to an ischemic hindlimb. Hypoxia-inducible factor 1 (HIF-1) is a posttranscriptionally regulated transcription factor, controlling several hypoxia-inducible genes [17]. Overexpression of HIF 1-related proteins might therefore induce a broader angiogenic response than overexpression of a single angiogenic factor.

Nitric oxide synthase (NOS), the enzyme responsible for endothelial NO production, might also be considered as a potential angiogenic factor, as both VEGF [18] and FGF [19] have been shown to modulate angiogenesis in part through the activation of NOS. Despite these exciting results two major caveats remain. First, the proof of concept of therapeutic angiogenesis in large animal models is a prerequisite for future gene therapy protocols in patients. Second, potential side effects associated with untoward angiogenesis need careful investigation.

Neangiogenesis is indeed implicated in physiological as well as pathological processes including tumor and metastatic growth, diabetic retinopathy, and atherosclerotic plaque rupture [20]. Inoue et al. [21] demonstrated that expression of VEGF and its receptors flt-1 and flk-1 correlated with the severity of atherosclerosis in human coronary arteries. Furthermore, inhibition of angiogenesis by specific anti-angiogenic molecules reduced plaque growth in an atherosclerotic mouse model [22], whereas local administration of FGF contributed to unfavorable arterial remodeling in injured coronary arteries [23], possibly due to enhanced adventitial angiogenesis and increased cellular proliferation. Another safety concern is the use of angiogenic factors in diabetic patients, who commonly suffer from diffuse coronary artery disease and are potential candidates for therapeutic angiogenesis. VEGF concentrations are increased in ocular liquid of patients suffering from diabetic retinopathy [24], and VEGF inhibition in an experimental model of ischemic retinopathy in mice was associated with less retinopathy [25]. Significant circulating levels of recombinant VEGF are detected after intramuscular [6] and intramyocardial [7] administration which may modulate unwanted angiogenesis at remote sites. Clearly, further basic research needs to clarify these safety issues, and expand our knowledge of therapeutic angiogenesis and vasculogenesis as new strategies for postnatal neovascularization [26].

3. Vasculoproliferative diseases

Gene therapy for restenosis also progressed from the experimental phase to the first clinical trials. To investigate if VEGF overexpression can accelerate re-endothelialization and thus reduce neoointima after local vascular injury, as demonstrated in a rabbit model [27], VEGF plasmid was locally delivered to patients after PTCA using the dispatch catheter [28]. Although no final results have been reported, the method seems feasible and safe in the short term. In another trial, antisense oligonucleotides against c-myc were used to prevent coronary restenosis in stented patients (ITALICS trial) [29]. Although successful in experimental porcine studies [30], the results could not be reproduced in patients in part due to methodological limitations. The delivery device could have been suboptimal for effective gene transfer in the vessel wall, and both dose–response...
and pharmacokinetic characteristics need a more detailed evaluation.

Two recent reviews have extensively covered the pros [31] and the cons [2] of gene therapy for restenosis. Despite major advances that have led to the first clinical trials, many hurdles remain. The difficulties to translate the excellent effect of many gene-based approaches in small animal models of vascular injury to larger, more relevant animal models and to patients relate to suboptimal delivery systems, and vector-associated toxicity.

Essential cell-cycle regulatory proteins have been targeted to prevent neointima formation, because smooth muscle cell (SMC) proliferation is a key component in the narrowing process in injured arteries [32]. In contrast, restenosis in patients is a complex biological phenomenon involving not only SMC proliferation, but also SMC migration and apoptosis, and matrix formation and degradation, which contribute to remodeling. Proteins with pleiotropic effects on vascular cell functions might have an advantage to better target the response to vascular injury in patients than is the case with cell-cycle inhibitory proteins. In this respect, overexpression of NOS [33], heme oxygenase (HO1) [34] and C-type natriuretic peptide (CNP) [35] have shown promising initial results, and deserve further investigation.

Local delivery via conventional interventional techniques remains the Achilles’ heel of vascular gene therapy because of low transfection efficacy. Surgical methods, including local dwelling, are cumbersome or impossible to perform routinely. Advenitial delivery of adenoviral vectors may cause less medial inflammation than intraluminal delivery [36], potentially reducing vector-induced inflammation, but requires surgical procedures or perforating needle catheters [37]. Few novel devices have emerged since a previous review in this journal more than 2 years ago [38]. Different approaches to optimize local gene delivery have been tested. In a comparative study, three intramural pressure-driven catheters and one mechanical intramural catheter were compared in a porcine coronary artery balloon-angioplasty model [39]. Three of the four catheters tested (Infusasleeve™, Crescendo™ and Infiltrator™) demonstrated comparable efficiency as reflected by vascular luciferase gene expression levels. Clearly, further development of interventional devices is essential to increase intracoronary gene transfer and to test the role of gene therapy for restenosis.

Another question relates to gene transfer efficacy and safety in complex, lipid rich atherosclerotic arteries. Adenovirus-mediated gene transfer of the marker gene human placental alkaline phosphatase was found equally effective in human atherosclerotic vessels and in normal vessels in organ culture [40]. Anti-neointimal strategies have been successful in atherosclerotic rabbits [41]. However, there might be some concern that adenoviral vectors may induce thrombosis in atherosclerotic arteries. Lafont et al. [42] found that adenoviral gene transfer induced thrombosis in atherosclerotic rabbit arteries, but not in normal arteries, although transfection efficiency was similar. Although NOS overexpression in balloon-injured porcine arteries reduces neointima [33], local NOS overexpression could paradoxically contribute to neointima formation in diseased [43], or balloon-injured [44] atherosclerotic arteries via the generation of toxic peroxynitrite [2]. In contrast, NOS overexpression in atherosclerotic rabbit arteries might reduce mural inflammation and lipid accumulation [45], and there are indications that NO reduces neointimal hyperplasia after balloon angioplasty in hypercholesterolemic rabbits [46].

The current widespread use of stents has virtually eliminated the problem of late constrictive remodeling, but remains associated with in-stent restenosis. In-stent restenosis is mainly caused by a SMC hyperproliferative response [47] and overexpression of NOS [48] or GAX [49] and chimeric DNA–RNA hammerhead ribozyme to proliferating cell nuclear antigen [50] were capable of significantly inhibiting stenosis in a porcine coronary injury model. Coating stents with DNA or viruses may provide an interesting alternative for local vascular gene transfer, obviating the need for sophisticated and expensive delivery devices. High transfection efficiencies were reported with a DNA-eluting polymer-coated stent in porcine coronary arteries [51]. Furthermore, biodegradable microporous intravascular stents can be impregnated with virus or DNA, providing a sustained-release local reservoir while still serving as an effective scaffold to the artery [52]. A few years ago, seeding of genetically modified cells on stents was proposed as a promising technique [53], but no intracoronary applications of this approach have been reported so far.

Preventing neointimal growth in venous bypass grafts or cardiac allografts might be a particularly attractive objective for gene therapy, mainly because of the easy surgical access during tissue prelevation [54]. Two years ago, the authors of a review on gene therapy for the prevention of vein graft failure [55] regretted the lack of interest for gene therapy in this field. Since then, pressure-mediated delivery of E2F decoy oligonucleotide was tested in vein grafts [56], and was associated with decreased primary vein graft failure [57]. Reduction of neointimal growth in vein grafts has also been reported after overexpression of a constitutively active retinoblastoma protein [58]. A different approach is to reduce SMC migration in vein grafts by overexpressing tissue inhibitors of metalloproteinases (TIMP) 1 and 2 [59,60], the elastase inhibitor elafin [61], or the senescent cell-derived inhibitor sdi-1 [62]. Coronary arteriosclerosis in a mouse cardiac transplantation model was prevented by transfer of antisense oligonucleotides against cyclin-dependent kinase CDK-2 [63].

Further insight in the arterial response to balloon-angioplasty and stent implantation in patients will be necessary to identify the best targets to reduce restenosis. Continuous improvements in PTCA and stent implantation...
procedures, and the availability of intracoronary radiation will need to be balanced against the added benefit of gene therapy for restenosis. Possible candidates for gene therapy in vasculoproliferative disease include in-stent restenosis, vein graft disease and transplant atherosclerosis.

4. Hypercholesterolemia and atherosclerosis

Atherosclerotic cardiovascular disease remains the leading cause of death in western countries. Inherited disorders of lipoprotein metabolism often lead to lipid levels that are difficult to control with conventional lipid-lowering drugs, making them particularly attractive targets for gene therapy. Four years ago, the first clinical study using ex vivo gene therapy for familial hypercholesterolemia was published [64]. Although significant reductions of LDL levels in three out of five patients tested were observed for four months after gene transfer, to date no other clinical expression of phospholamban [78] or published [64]. Although significant reductions of LDL potentiated myocardial contraction by bypassing the de-vivo gene therapy for familial hypercholesterolemia was transfer of a vasopressin receptor in cardiomyocytes therapy. Four years ago, the first clinical study using ex proteins with a positive inotropic effect. Adenoviral gene making them particularly attractive targets for gene-based approaches for heart failure are focusing on.

Gene therapy for atherosclerosis and hyperlipidemia has been focusing on modulating lipoprotein metabolism. Successful attempts have been made to elevate HDL [65], and to decrease LDL [66], VLDL [67], and triglyceride levels [68]. The effect of gene therapy on lipid profiles in transgenic or knock-out mice lacking key components of the lipoprotein metabolism has been amply reviewed [69]. Gene-based techniques have also contributed to a better understanding of the pathogenesis of atherosclerosis [70]. More recently, research has shifted to explore the effect of lipoprotein gene transfer on the development of the atherosclerotic process itself [71]. Although larger animal models for atherosclerosis are under investigation, a proper interpretation of the effects of local genetic interventions on the atherosclerotic process, plaque stability and plaque progression remains very difficult. This is mainly due to the large variation and the unpredictability of lesions in these animals. Nevertheless, gene-based strategies for atherosclerosis and its complications will require experimental scrutiny in relevant animal models before they can be considered for clinical applications.

Alternative approaches to reduce atherosclerosis and its complications include the reduction of risk factors, e.g. the inhibition of apolipoprotein(a) expression, a known risk factor for atherosclerosis [72]. Novel non-lipoprotein-based strategies, including overexpression of NOS with subsequent reduction of inflammatory cell infiltration and lipid accumulation, might also be of interest [45]. The use of gene therapy to stabilize arterial plaques might be a promising approach [73]. However, intravascular administration of the gene encoding β-galactosidase to brachial arteries of hypercholesterolemic monkeys increased vessel wall inflammation and was associated with progression of early atherosclerotic lesions [74]. This might have been caused by the vector itself, or by an immunological reaction to the transgene product. Gene transfer into the atherosclerotic wall to reduce plaque progression or plaque rupture may therefore require less immunogenic vectors [75].

5. Myocardial diseases

The incidence of congestive heart failure is increasing [76] and despite advances in pharmacological treatment, the associated morbidity and mortality remain high. Therefore, gene-based strategies to improve cardiac function and overall clinical outcome might be particularly useful for these patients.

Gene-based approaches for heart failure are focusing on proteins with a positive inotropic effect. Adenoviral gene transfer of a vasopressin receptor in cardiomyocytes potentiated myocardial contraction by bypassing the desensitized β-adrenergic receptor signaling [77], and over-expression of phospholamban [78] or β2-adrenergic receptor [79] improved ventricular function in rodent hearts. Gene transfer vectors with extended transgene expression profiles are required to effectively target heart failure. In this respect, intramyocardial or intracoronary injection of recombinant adenovirus-associated vector encoding β-galactosidase resulted in significant transgene expression levels for up to 8 weeks after the intervention, without evidence of myocardial inflammation or necrosis [80].

Myocardial infarction is a recent target for gene-based strategies, and reduction of reperfusion injury by over-expressing antioxidative proteins might be a valuable approach. Overexpression of extracellular superoxide dismutase (ec-SOD) attenuated stunning in ischemic rabbit hearts [81], and adenovirus-mediated cardiac gene transfer of the antioxidant proteins SOD and catalase reduced contractile dysfunction after ischemic reperfusion in the neonatal mouse heart [82]. Delivery of decoy double stranded DNA against NFκB, a transcriptional factor that regulates cytokine and adhesion genes, reduced the extent of myocardial infarction after reperfusion in the rat [83]. However, gene transfer directly into infarcted myocardium suffers of low transfection efficiency when compared to non-infarcted normal myocardium [84]. Further experiments will have to determine the optimal delivery method and time of delivery of candidate genes for the reduction of necrosis after myocardial infarction.

A completely different genetic approach to the treatment of heart failure and myocardial infarction, is transfer of ex vivo modified cells to the myocardium, as reviewed before [85]. Primary cells were forced to differentiate into muscle cells after overexpression of MyoD, a transcription factor that drives myogenesis in non-muscle cells [86]. This could in theory modify the phenotype of the failing heart, provided that a sufficient number of cells can be implanted without prohibitive toxicity. These obstacles have thus far limited the applicability of the technique in large animal models.
Gene therapy for inherited myocardial diseases still suffers from many of the obstacles pointed out 2 years ago [87]. Targeting a sufficient number of myocytes remains a challenge, and several approaches are being contemplated to improve efficacy. Intracorony injection is a fairly straightforward approach, but leads only to very limited transduction, with only 0.3% of the cells transsected with an adenoviral vector [88]. Pericardial delivery is under investigation and has been combined with matrix degrading proteins which facilitate the penetration of the visceral pericardial barrier and markedly increase efficiency [89]. Also, retrograde delivery via the sinus venosus during occlusion of the LAD has been shown to transduce 30–50% of cardiomyocytes in the LAD region [90].

Recently, gene-based approaches have also been proposed for the treatment of arrhythmias, both for acquired rhythm abnormalities (e.g. in heart failure or myocarditis [91]) and for inherited diseases (e.g. the long QT syndromes [92] and familial atrial fibrillation [93]). Improved delivery systems and vectors with longer and more stable expression patterns will allow future investigations in this field.

6. Thrombosis

In a review on gene therapy for arterial thrombosis [94], the first experiments to deliver antithrombotic genes (including hirudin, urokinase and cyclooxygenase) in injured arteries were described. To date, several new genes have been studied for their ability to reduce thrombosis. Adenoviral mediated overexpression of tPA [95], thrombomodulin [96], or tissue factor pathway inhibitor [97] reduced arterial thrombosis in injured rabbit arteries. These genetic approaches need to be weighed against developments in antithrombotic molecules (GP IIbIIIa antagonists), which have significantly improved the safety/efficacy profile of these drugs in patients suffering from acute coronary events [98]. Certainly, if local overexpression of antithrombotic gene products will further decrease bleeding complications associated with current systemic therapy and retain therapeutic efficiency, this approach might be a valuable alternative.

7. Hypertension

Genetic strategies based on inhibition of components of the renin–angiotensin system are currently emerging to treat systemic hypertension [99,100]. Intravenous injection of the gene encoding atrial natriuretic peptide (ANP) not only attenuated hypertension in rats, but also decreased cardiac hypertrophy and renal injury [101]. While gene transfer is certainly feasible and has shown proof of principle [102], it seems nevertheless unlikely that it will replace pharmacological therapy in the near future, given the multifactorial etiology of the disease and the very effective pharmacological therapy.

In contrast, gene therapy for pulmonary hypertension might offer novel perspectives as pharmacological treatment options are often limited. Adenovirus-mediated delivery of constitutive NO Synthase via aerosol decreased both acute [103] and chronic hypoxia-induced pulmonary hypertension in rats [104]. As extended transgene expression is required for the treatment of chronic pulmonary hypertension, and because of uncertainties surrounding inflammatory reactions and immunogenicity of current vectors, improvements in vector design are necessary before considering clinical applications.

8. New vectors

To date, DNA and oligonucleotide-based treatments have been very popular, probably due to less safety concerns as compared with viral vectors, and have been used in the first clinical trials. However, there is evidence that adeno-associated virus-mediated delivery of E/Gene (including hirudin, urokinase and cyclooxygenase) in injured arteries was described. To date, several new genes have been studied for their ability to reduce thrombosis. Adenoviral mediated overexpression of tPA [95], thrombomodulin [96], or tissue factor pathway inhibitor [97] reduced arterial thrombosis in injured rabbit arteries. These genetic approaches need to be weighed against developments in antithrombotic molecules (GP IIbIIIa antagonists), which have significantly improved the safety/efficacy profile of these drugs in patients suffering from acute coronary events [98]. Certainly, if local overexpression of antithrombotic gene products will further decrease bleeding complications associated with current systemic therapy and retain therapeutic efficiency, this approach might be a valuable alternative.

Although clearly more effective than naked DNA, viral vectors still suffer from local and systemic toxicity. Replication-defective adenoviruses are generated using a helper cell-line that provides the missing E<sup>3</sup> gene function. Due to possible recombinant events between sequences in the vector and in the packaging helper cell-line, this method does not entirely exclude the generation of replication-competent adenoviral clones (RCA) during the manufacturing process. As RCA may contribute significantly to adenovirus-related local and systemic toxicity, the development of a new helper cell-line for the generation of virus batches free of RCA has been a major step towards future clinical use of these vectors. Because RCA have been implicated in the genesis of cardiomyopathies, previous caveats on the use of viral vectors in myocardial disease [87] will need to be reconsidered. Thus vectors with additional deletions of adenoviral gene sequences (E<sub>2A</sub>/E<sub>4</sub> [108], E<sub>r</sub>/E<sub>4</sub> [109], E<sub>r</sub>/E<sub>1</sub>/E<sub>4</sub> [110]) or vectors with all of the viral genes deleted (‘gutless’ vectors) have been constructed [111] and tested in animals, often with conflicting results. However, proper engineering
of viral genes will likely result in adenoviral vectors with low toxicity and immunogenicity, and which will allow prolonged expression and repeated administration.

Adenovirus-associated virus (AAV)-derived vectors have been proposed as a valuable alternative for cardiovascular gene therapy. AAV-derived vectors have some advantages over adenoviral vectors, mainly because of their non-pathogenic nature and absence of inflammation, allowing stable, long-term expression [112]. Expression of a reporter gene was observed in rat carotid arteries up to 6 months after infection with AAV [113]. On the other hand, the AAV genome is small, only allowing room for about 4.8 kb of added DNA, and the difficulties of reproducible amplification procedures to yield high titer AAV-derived vectors have limited this far the introduction of this vector in cardiovascular disease models. Nevertheless, efforts to improve infectious titer and yield [114] have initiated the first AAV cardiovascular gene therapy protocols in vitro [115] and in vivo [80,116].

Retroviral vectors integrate into the host cell’s genome and result in a more stable and longer expression. They can only infect dividing cells and can theoretically induce malignant transformation by their random integration in the host cell DNA. As for adenoviruses, one of the major concerns with retroviral vectors is the possibility that a replication-competent retrovirus is generated during the manufacturing process. Nevertheless, retroviral vectors are currently used for cardiovascular gene therapy protocols [99,117], and the advent of lentiviral vectors, which can infect non-dividing cells [118] is expected to facilitate its application for cardiovascular diseases. This is of particular interest for cardiovascular gene therapy where long-term expression is desirable. An alternative to increase retroviral vector transfer efficiency is to stimulate cell proliferation in the target tissue. A limited partial liver resection to stimulate the rate of hepatocyte turn-over was performed in combination with portal vein injection of cytotoxic thymidine kinase gene followed by ganciclovir treatment. This combined suicidal gene transfer and surgical intervention resulted in improved hepatic uptake and expression of the desired transgene [119].

Directing gene transfer vectors to the target cells exclusively might also reduce toxicity and increase efficiency. This can be done physically with microspheres carrying vectors. Intravenous administration of oligonucleotides against c-myc bound to microbubbles, followed by thoracic ultrasound-mediated destruction of these bubbles, prevented stenosis in balloon-injured porcine arteries [120]. Another approach is the construction of new vectors with specific promoters which regulate transgene expression, as already described two years ago [121]. These new promoters may include cell-specific promoters including the smooth muscle actin promoter [122] or the α-myosin heavy chain promoter [123] to target the vascular SMCs and cardiomyocytes respectively. Alternatively, vectors can incorporate promoters with conditional expression including hypoxia-induced [124] or shear stress-sensitive promoter sequences, or the viral vector envelope can be modified to increase tissue specificity. The retroviral envelope has been modified to incorporate the high affinity collagen-binding domain from von Willebrand factor [125], targeting the infection to the subendothelial matrix. As vascular injury exposes collagen in the vessel wall, this vector might be particularly interesting to target damaged arteries.

9. Conclusions

Cardiovascular gene therapy has reached the clinical realm, and is likely to stay there. New clinical studies using gene therapy in patients with cardiovascular disease are likely to emerge in the following years, particularly in the fields of restenosis, heart failure and angiogenesis. Further insights in cardiovascular molecular biology will at the same time provide new potential targets for gene-based strategies. Effective and safe delivery methods, together with new vectors for target-specific binding or synthetic formulations with high efficiency and safe profile will undoubtedly lead to wider applicability of gene therapy in cardiovascular disease. Despite our present lack of knowledge, intensified basic and applied research will certainly bring the first successes of cardiovascular gene-therapy protocols in the years to come.

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