Gene Therapy for Cardiovascular Disease
A Case for Cautious Optimism

Rohit Khurana, John F. Martin, Ian Zachary

Abstract—There is currently intense interest in the development of gene therapy for cardiovascular disease. The stimulation of therapeutic angiogenesis for ischemic heart disease has been one of the areas of greatest promise. Encouraging results have been obtained with the angiogenic cytokines vascular endothelial growth factor (VEGF) and basic fibroblast growth factor in animal models, leading to clinical trials in ischemic heart disease. VEGF also has therapeutic potential in a second area of cardiovascular gene therapy, the enhancement of arterioprotective endothelial functions to prevent postangioplasty restenosis and bypass graft arteriopathy. The endothelial cell growth and survival functions of VEGF promote endothelial regeneration, whereas VEGF-induced endothelial production of NO and prostacyclin inhibits vascular smooth muscle cell proliferation. Inhibition of neointimal hyperplasia may also be achieved by gene transfer of endothelial NO synthase (eNOS), PGI synthase, or cell cycle regulators (retinoblastoma, cyclin or cyclin-dependent kinase inhibitors, p53, growth arrest homeobox gene, fas ligand) or antisense oligonucleotides to c-myb, c-myc, proliferating cell nuclear antigen, and transcription factors such as nuclear factor κB and E2F. An improved understanding of etiologically complex pathologies involving the interplay of genes and the environment, such as atherosclerosis and systemic hypertension, has led to the identification of new targets for gene therapy, with the potential to alleviate inherited genetic defects such as familial hypercholesterolemia. The use of vasodilator gene overexpression and antisense knockdown of vasoconstrictors to reduce blood pressure in animal models of systemic and pulmonary hypertension offers the prospect of gene therapy for human hypertensive disease. The renin-angiotensin system has been the target of choice for antihypertensive strategies because of its wide distribution and additional effects on fibrinolytic and oxidative stress pathways. Gene therapy in cardiovascular disease has an exciting future but remains at an early stage. Further developments in gene transfer vector technology and the identification of additional target genes will be required before its full therapeutic potential can be realized. (Hypertension. 2001;38:1210-1216.)

Key Words: cardiovascular gene therapy ■ angiogenesis ■ atherosclerosis ■ hyperlipidemia

Coronary artery disease remains one of the leading causes of morbidity and mortality in developed nations and is projected to be the leading cause of death in the developing world by 2010.1 The relative accessibility of the vasculature and the myocardium makes them ideal targets for gene therapy, and advances both in understanding the molecular basis of disease and in gene transfer technology now make therapeutic gene delivery to diseased organs an increasingly realistic goal. Though many cardiovascular diseases are theoretically amenable to this approach, translating the fruits of basic scientific progress into clinical benefits remains a formidable challenge. In this review, we consider the prospects for gene therapy in 4 areas: (1) therapeutic angiogenesis for ischemic heart disease, (2) the prevention of vasculoproliferative disease after intervention by nonsurgical (postangioplasty and in-stent restenosis) or surgical procedures (failure of bypass grafts, coronary allografts, vascular prostheses, and anastomoses), (3) systemic and pulmonary hypertension, and (4) inherited diseases of lipid metabolism.

Factors Influencing Successful Gene Therapy in the Cardiovascular System
Apart from the therapeutic gene itself, gene-based medicine requires 2 components: (1) a DNA vector and/or packaging system that enables the gene to enter the target cell and be efficiently expressed and (2) a means of gene delivery to the organ of choice.

Though the maximization of gene transduction efficiency is usually regarded as a desirable goal, the greatest level of expression attainable may not necessarily be the most logical choice for a given therapeutic situation. High-efficiency expression of gene products that act intracellularly may be desirable, but therapeutically useful effects of gene products that are secreted or that mediate production of secreted products may be achieved at lower levels of expression. Indeed, high-efficiency expression of either intracellularly or extracellularly directed genes may be a disadvantage if overproduction of the product has harmful side effects. As discussed below, this consideration may be particularly rele-
vant to the use of angiogenic cytokines, such as vascular endothelial growth factor (VEGF).3

The site and physical means of administration affect the efficacy of a given mode of delivery, and both these variables may be key determinants of therapeutic outcome. The routes of delivery to the cardiovascular system are endoluminal (eg, using a gene delivery catheter during angioplasty), periventricular, and direct injection into the myocardium, coronary artery, pericardium, or ventricle. Intravenous or subcutaneous administration are options for protein therapy only. The choice will be influenced by considering the cell type in which it is most desirable that the gene be expressed, the ultimate therapeutic target of the gene product, the nature of the disease or clinical setting being targeted, and the risk posed by systemic diffusion of the therapeutic gene. For example, it might appear preferable that eNOS is delivered to the endothelium, even though the target is inhibition of VSMC proliferation, because endothelial cells have the requisite cellular machinery to produce NO efficiently. Cell type–specific targeting of eNOS is unlikely to be a key issue; however, as the goal is to increase NO production in the local milieu, and systemic leakage will probably not pose a risk. In contrast, inhibitors of the cell cycle, which act intracellularly to block VSMC proliferation, should preferably be targeted directly to VSMCs. In the case of angiogenic cytokines such as VEGF, the danger posed by gene diffusion leading to pathophysiological neovascularization argues in favor of localized and restricted gene delivery.

**Myocardial Angiogenesis**

A significant proportion of patients with ischemic heart disease either cannot undergo coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA).3 Many of these individuals have residual symptoms or myocardial ischemia despite maximal medical therapy. The angiogenic cytokines VEGF and basic FGF (FGF-2) have been used with considerable success to increase blood supply in various animal models of peripheral limb and myocardial ischemia.4–7 Based on this impressive body of experimental work, initially promising results were obtained from administration of VEGF to patients with ischemic heart disease.8 These studies were, however, very small and without placebo controls (summarized in Table 1).22,24,25,26

The first sizeable, controlled clinical study of VEGF (VEGF in Ischemia for Vascular Angiogenesis [VIVA]) for therapeutic angiogenesis used intracoronary followed by intravenous VEGF-A165 protein administration versus placebo in 178 patients with ischemic heart disease who were not optimal candidates for CABG or PTCA.9 Treated patients failed to meet the primary endpoint of improved exercise tolerance and symptomatic improvement in angina class, and there were no significant differences in nuclear perfusion or angiography after 60 or 120 days. Furthermore, the highest VEGF dose tested in the VIVA trial, 50 ng · kg–1 · min–1, was not found to be effective in inducing angiogenesis in a porcine ameroid model study.10 Clinical use of FGF-2 is also based on a large amount of animal data.6,7 Intracoronary administration of recombinant FGF-2 to 52 patients with coronary artery disease resulted in prolonged exercise treadmill times, but these findings should be interpreted cautiously given the absence of a “treated” placebo protein group.11 Interestingly, magnetic resonance imaging myocardial perfusion studies showed a significant improvement in left ventricular contractile function, but nuclear perfusion imaging studies did not. Major adverse cardiac events (death or myocardial infarction) at 60 days were relatively and inexplicably high, occurring in 5 out of 52 treated patients. The same group also reported the perivascular application of recombinant FGF-2 at thoracotomy, to improve revascularization in patients undergoing CABG.12 The safety and feasibility of this approach with a 100 mg dose was confirmed at the 90-day evaluation using both nuclear and MRI perfusion imaging, but whether it proves to be a valuable adjunct in the long-term awaits follow-up. The conclusions of these findings led to the initiation of the FIRST trial, a 337-patient double-blinded phase II trial that examined 3 different intracoronary doses of FGF-2 protein.

### Table 1. Summary of Gene- and Protein-Based Growth Factor Therapy in Clinical Studies/Trials Conducted In Patients With Severe Coronary Artery Disease

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vector or Protein</th>
<th>Mode of Delivery</th>
<th>Patients</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-A</td>
<td>Naked DNA</td>
<td>Intramyocardial injection via thoracotomy</td>
<td>5, 20</td>
<td>Lasordo et al, Symes et al</td>
</tr>
<tr>
<td>LacZ/VEGF</td>
<td>Liposome/adenovirus</td>
<td>Infusion-perfusion</td>
<td>65</td>
<td>Laitinen et al</td>
</tr>
<tr>
<td>VEGF-A 21</td>
<td>Adenovirus</td>
<td>Intramyocardial injection during bypass</td>
<td>21</td>
<td>Rosengart et al</td>
</tr>
<tr>
<td>FGF-4</td>
<td>Adenovirus</td>
<td>Intracoronary injection</td>
<td>100</td>
<td>Hammond et al</td>
</tr>
<tr>
<td>VEGF-C</td>
<td>Naked DNA</td>
<td>Intramyocardial injection</td>
<td>18</td>
<td>Ylä-Herttula et al</td>
</tr>
<tr>
<td>FGF-1</td>
<td>Recombinant protein</td>
<td>Intramyocardial injection during bypass</td>
<td>20</td>
<td>Schumacher et al</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Recombinant protein</td>
<td>Peri-adventitial delivery with biodegradable matrix</td>
<td>24</td>
<td>Laham et al</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Recombinant protein</td>
<td>Intracoronary injection</td>
<td>52</td>
<td>Laham et al</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Recombinant protein</td>
<td>Intravenous injection</td>
<td>14</td>
<td>Laham et al</td>
</tr>
<tr>
<td>h-VEGF</td>
<td>Recombinant protein</td>
<td>Intracoronary followed by intravenous injection (VIVA trial)</td>
<td>178</td>
<td>Henry et al</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Recombinant</td>
<td>Intracoronary injection (FIRST trial)</td>
<td>337</td>
<td>Post et al</td>
</tr>
</tbody>
</table>
versus placebo controls. The overall results at the 90-day follow-up were disappointing, with no overall improvement in the size of the ischemic territory and treadmill times.

Though the VEGF and FGF-2 trials failed to translate the optimism generated by animal studies into clinical benefit, they leave unresolved the question of the efficacy of gene versus protein therapy. Gene therapy theoretically requires only a single dose to produce a sustained local presence of the desired growth factor, and this may be preferred to the repeated administration of a protein product, which, as the pharmacokinetics of VEGF protein delivery show, is rapidly cleared from the plasma. The results of gene transfer strategies in humans have yet to be reported, but a multicenter phase I/II placebo-controlled double-blind clinical trial of intracoronary administration of adenoviral FGF-4 in patients with stable class II/III angina is planned.

Reliable, objective, and more efficacious endpoints need to be defined and validated for the evaluation and long-term monitoring of angiogenic and future arteriogenic therapies. Current clinical trials use the New York Heart Association classification for symptomatic assessment and the ongoing dependence on medication. Noninvasive techniques such as perfusion scintigraphy, positron emission tomography, myocardial contrast echocardiography, and SPECT (single photon emission computed tomography) imaging allow assessment of perfusion in the region of interest. Unfortunately, these techniques are at present unable to assess flow in subendocardial regions, which are known to be preferential regions for collateral vascular growth in humans. Repeated coronary angiography allows serial visualization of collateral arteries, but its invasive nature diminishes its value as a clinical tool. It will also be important to determine whether the theoretical benefits conferred by growth factor gene therapy result not only in the amelioration of symptoms and improved follow-up imaging but also delayed mortality.

In addition to the formidable problem of endpoint assessment, the use of FGFs and VEGF for therapeutic angiogenesis is problematic for several reasons. The maximal intravenous or intracoronary doses of both VEGF and FGF are limited by their propensity to induce clinically significant hypotension. It is also uncertain whether the ischemic, peri-ischemic, or nonischemic myocardial zone alone or in combination should be targeted. VEGF-induced angiogenesis may accelerate occult neoplastic disease or atherosclerosis, a problem highlighted by the finding that sustained VEGF expression causes hemangiomias after VEGF-transfected myoblast implantation in mice. The debate on the use of VEGF therapy for cardiovascular disease was plunged deeper into controversy by the provocative finding that administration of a single bolus of VEGF accelerated atherosclerotic plaque formation in an ApoE knockout mouse model. The relevance of the ApoE knockout mice findings for the human clinical experience has been called into question, however. To date, several hundred patients have been treated with VEGF and other angiogenic cytokines with no reported increase in symptomatic disease.

### Table 2. Candidate Target Genes for Cardiovascular Therapy

<table>
<thead>
<tr>
<th>Therapeutic Arteriovascular Indication</th>
<th>Post Angioplasty Stenosis/In-Stent Restenosis/Bypass/Allograft Failure</th>
<th>Arterioprotection</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF A, B, C, D, E</td>
<td>VEGF A, C</td>
<td>VEGF A, C</td>
</tr>
<tr>
<td>FGF 1, 2, 4, 5</td>
<td>FGF</td>
<td>eNOS</td>
</tr>
<tr>
<td>Angiopoietin 1, 2</td>
<td>PDGF</td>
<td>PGI synthase</td>
</tr>
<tr>
<td>HGF, PDGF, PIGF</td>
<td>eNOS, INOS</td>
<td>COX</td>
</tr>
<tr>
<td>MCP-1</td>
<td>PGI synthase</td>
<td></td>
</tr>
<tr>
<td>MMP</td>
<td>TIMPs</td>
<td></td>
</tr>
<tr>
<td>eNOS, INOS</td>
<td>Cytotoxins: TK, CD</td>
<td></td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Cell cycle regulators: NFαx and E2F decoys</td>
<td></td>
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<tr>
<td></td>
<td>Rb protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p21, p27</td>
<td></td>
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<tr>
<td></td>
<td>p53</td>
<td></td>
</tr>
<tr>
<td>c-myb, c-myc, cd2, cdk2, PCNA</td>
<td></td>
<td></td>
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<tr>
<td>Homeobox genes (gax)</td>
<td></td>
<td></td>
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<tr>
<td>Vascular signal transducers: ras</td>
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<tr>
<td>ApoE</td>
<td></td>
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<tr>
<td>Hyperlipidaemia</td>
<td></td>
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<tr>
<td>Hyperlipidaemia</td>
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</tr>
<tr>
<td>LDLr, VLDlr</td>
<td>eNOS</td>
<td>eNOS, INOS</td>
</tr>
<tr>
<td>ApoE</td>
<td>RAS: AT-1r, ACE</td>
<td>PGI synthase</td>
</tr>
<tr>
<td>Lipoprotein lipase, hepatic lipase</td>
<td>Kallikrein</td>
<td>CGRP</td>
</tr>
<tr>
<td>HDL:</td>
<td>ANP</td>
<td></td>
</tr>
<tr>
<td>apoA-I, II, IV</td>
<td>Adrenomedullin</td>
<td></td>
</tr>
<tr>
<td>Enzymes: CETP, LCAT</td>
<td></td>
<td></td>
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<tr>
<td>Receptor-SR-B1</td>
<td></td>
<td></td>
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<tr>
<td>Lp(a)</td>
<td></td>
<td></td>
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<tr>
<td>VCAM, ICAM</td>
<td></td>
<td></td>
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<tr>
<td>Antioxidative enzymes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| HGF indicates hepatocyte growth factor; PDGF, platelet derived growth factor; PIGF, placenta growth factor; MCP-1, monocyte chemotactic protein-1; MMP, matrix metalloproteinase; NOS, nitric oxide synthase; HIF-1α, hypoxia inducible factor-1α; TIMPs, tissue inhibitors of metalloproteinases; TK, thymidine kinase; CD, cytochalasin D, Rb, retinoblastoma; FasL, Fas ligand; and CDX, cycloxygenase.

The diversity of cell types and cytokines involved in arteriogenesis suggest that administration of a single “magic bullet” gene is unlikely to regulate all the events necessary for restorative collateral coronary artery formation (Table 2). Effective therapeutic arteriogenic strategies may need to be based on combinatorial gene (or protein) delivery approaches.

### Vasoproliferative Disease and Arterial Protection

The durability of angioplasty, bypass, and endarterectomy of peripheral and coronary vessels is limited by intimal hyperplasia of phenotypically modified smooth muscle cells and accumulation of matrix proteins within media. Progressive encroachment of the vessel lumen results in stenosis and/or...
occlusion and therefore ischemic injury in the downstream tissue.

Gene therapy strategies directed at inhibiting VSMC proliferation and migration, by interfering with intracellular transduction pathways, have been shown to be effective in retarding the development of intimal disease. Promising results emerged from the randomized, controlled PREVENT trial, which, using small patient numbers, showed a reduced rate of lower extremity venous bypass graft failure in response to transfection with an antisense oligonucleotide to E2F transcription factor, a protein necessary for activating several genes required for smooth muscle cell proliferation. Other antisense constructs directed against cell cycle regulators—such as c-myb, c-myec, cdc-2, cdk-2, proliferating cell nuclear antigen, and retinoblastoma protein or blockade of intracellular signal transducers (eg, ras and Raf kinase)—have also diminished intimal thickening with varying degrees of efficacy in animal models of restenosis. Conversely, overexpression of the cyclin-dependent kinase inhibitor, p21, has similarly been shown to reduce neointima formation.

VSMC apoptosis is a prominent feature of the response to arterial injury and neointima formation. However, the pathogenic role of apoptosis in the natural history of the atherosclerotic plaque has not been established. It has been proposed that VSMC apoptosis predisposes to plaque rupture and subsequent myocardial infarction. Conversely, once VSMCs migrate into the intimal space during restenosis or graft neointimal hyperplasia, it is argued that an ongoing response to transfection with an antisense strategy directed at downregulating the expression of the anti-apoptotic Bcl-xL protein. It may be concluded that a more refined level of understanding of apoptosis regulation within the vasculature and its role in atherosclerotic disease is probably needed before useful trials can be initiated.

The approaches considered so far in this section have focused on direct inhibition of VSMC proliferation either by compromising pathways essential for growth or by stimulating pathways that inhibit growth or cause cell death. The effectiveness of this approach may be limited by the fact that many of the candidate targets act intracellularly, therefore requiring a high efficiency of gene transfer. An alternative approach is to augment endothelial functions which themselves mediate inhibition of VSMC proliferation and neointimal accumulation. The potential value of such a strategy is demonstrated by the findings that gene transfer of eNOS and PGI synthase inhibit neointima formation presumably via enhanced synthesis of, respectively, NO and PGI2, both potent inhibitors of VSMC proliferation. In support of this hypothesis, periadventitial gene transfer of VEGF has been demonstrated to reduce neointima formation in a nonendothelial injury animal model via a partly NO-mediated mechanism. In the balloon-injury iliac artery rabbit model, recombinant VEGF protein was shown to prevent intimal thickening and stent thrombosis. Local VEGF gene delivery strategies using a periadventitial collar may have therapeutic value by preventing vein graft stenosis in CABG or other grafts. Similarly, intravascular transfer of VEGF may be a useful adjunct in angioplasty or stent insertion by promoting arterioprotective functions and accelerating reendothelialization.

Atherogenesis

Inherited genetic defects in lipid metabolism, such as functional LDL and VLDL receptor deficiency culminate in premature IHD. Such defects may be amenable to both liver-directed receptor gene transfer in vivo and implantation of gene transfected autologous hepatocytes. The first clinical trial used transplantation of autologous hepatocytes that had been genetically corrected with a recombinant retrovirus carrying the LDL receptor and a 6% to 23% reduction in plasma LDL levels was achieved in 3 of the 5 treated patients. Lipoprotein lipase and hepatic lipase are both important for lipoprotein metabolism, and their deficiencies are also amenable to replacement by liver- or muscle-targeted gene transfer. The putative atherogenic lipoprotein(a), comprises a particle of LDL with its lipid and apoB-100 moiety attached to apoprotein(a). Apoprotein(a) levels can be lowered by ribozymes designed to inhibit its synthesis. High concentrations of apolipoprotein (apo) B100 may be lowered by gene transfer with the catalytic subunit of apoB mRNA-editing enzyme.

Susceptibility to atherosclerosis in humans is inversely correlated to the concentration of plasma HDL, and raising HDL concentration has been the focus of intense research. Transgenic mice overexpressing ApoA-1 have increased HDL cholesterol levels, and ApoA-1 was one of the first genes targeted for overexpression to generate a therapeutic response. Transgenic expression of human ApoA-1 into C57BL/6 mice by adenoviral gene transfer persisted for up to 10 weeks and resulted in a strong antiatherogenic effect. Scavenger receptor B-1 (SR-B1) is a multifunctional receptor that mediates HDL cholesterol transport into target tissues expressing SR-B1 (adrenal gland, liver) and is a potential potent regulator of HDL levels in vivo. A knockout of SR-B1 led to a doubling of plasma HDL cholesterol levels and increased particle size. ApoE has an important role in triglyceride-rich VLDL and remnant metabolism, and so type III hyperlipoproteinemia could hypothetically be treated with apoE gene transfer. It has yet to be demonstrated in patients with apoE deficiency whether liver- or muscle-directed gene transfer would be efficacious. Overall, conventional transgenic and knockout technologies have provided major insights into dyslipidemias and their predisposition to atherogenesis. Bridging the gap between the laboratory and the clinic is a prospect for the not too distant future.

Systemic Hypertension

Although systemic hypertension is a major risk factor for stroke, ischemic heart disease, cardiac arrhythmia, peripheral vascular disease, and progressive renal damage, only a small percentage of hypertensive patients have proven genetic abnormalities. The complex and multifactorial etiology has led some investigators to question the feasibility of gene therapy for hypertension (Figure). Overexpression of the vasodilator genes atrial natriuretic peptide, kallikrein, and its role in atherosclerotic disease is probably needed before useful trials can be initiated.
adrenomedullin,\(^{59}\) and eNOS,\(^{60}\) via transfer of naked DNA or viral delivery systems, caused blood pressure lowering in the 20 to 30 mm Hg range over a 6- to 12-week period after delivery to various rat models. While these studies provided support for the usefulness of vasodilator overexpression for the control of hypertension, inhibition of vasoconstrictor activity has also been a focus for research. The widely distributed renin-angiotensin system (RAS) is essential in both the normal regulation of blood pressure and the pathophysiology of chronic high blood pressure and has been the target of choice for antisense gene therapy to reverse established hypertension. Traditional pharmacological agents that target the RAS, such as ACE inhibitors and angiotensin receptor antagonists, are proven antihypertensive medications\(^{61}\) and provide a point of comparison against which to gauge the efficacy of antisense gene therapy. Intracardiac injections of viral particles containing antisense oligonucleotides to angiotensinogen or angiotensin II type I receptor into the adult spontaneously hypertensive rat (SHR) resulted in a 30 to 60 mm Hg reduction in blood pressure and was sustained for 36 days.\(^{62}\) This was accompanied by improved vascular reactivity, reversal of endothelial dysfunction, and ion channel dysfunction in renal resistance arterioles. Retroviral-mediated ACE antisense delivery in SHR elicited a modest antihypertensive response lasting up to 100 days.\(^{63}\) An optimistic evaluation of these results is tempered by the transient and modest nature of the therapeutic response. This may reflect the multifactorial nature of the disease as well as limited gene transfer efficiency. Despite the etiological complexity of systemic hypertension, the results of animal experiments provide some support for the use of targeted gene disruption or overexpression as a therapeutic approach to this condition.

**Primary Pulmonary Hypertension**

Primary pulmonary hypertension is a progressive disease characterized by intimal hyperplasia and in situ thrombosis, which results in raised pulmonary vascular resistance and diminished right heart function. Overexpression of vasodilator genes such as eNOS, prepro–calcitonin gene-related peptide (CGRP) and PGI synthase have produced promising results in animal models. In mice subjected to chronic hypobaric hypoxia, a potent stimulus for the development of pulmonary hypertension through vascular remodeling, transgenic overexpression of PGI synthase, and hence PGI2 production was found to have a lower right ventricular pressure than controls.\(^{64}\) The neuropeptide, CGRP, is expressed in nerve fibers of lung airways, and its receptors are highly expressed by VSMC in the pulmonary vasculature. Transfer of an adenoviral CGRP gene to lung epithelium by inhalation attenuated the increase in pulmonary vascular remodeling in mice exposed to chronic hypoxia (10% O\(_2\)) with a reduction in pulmonary artery resistance and pressure and a decrease in right ventricular mass.\(^{65}\) Similarly, delivery of aerosolized adenoviral inducible NO synthase enhanced pulmonary NO production and reduced hypoxic pulmonary vascular remodeling and hypertension in rats.\(^{66}\) The phenotype of the eNOS knockout mouse is characterized by pulmonary hypertension, which is reversed when the eNOS gene is delivered back to the lung.\(^{67}\)

**Conclusions and Future Directions**

The ability of gene disruption and gene delivery to alleviate pathophysiological changes in diverse animal models of cardiovascular diseases is now well documented. At present, however, neither interference of target gene expression nor the transfer of therapeutic genes is used clinically for cardiovascular disease, and the biggest challenge remains to translate basic experimental findings into clinical benefits.

An important issue in most gene therapeutic approaches is the desirability for more precise gene delivery to the target tissue or cell type. Tissue-specific gene expression can be enhanced by the use of cardiac myocyte-specific promoters, such as mlc-v (ventricle-specific myosin light chain-2) and aa-mhc (aa-mysin heavy chain) in conjunction with an intracardiac cavity injection technique\(^{68}\) or the cardiac tropinin T (cTNT) gene promoter.\(^{69}\) Local transfer enhances the efficiency of gene therapy by concentrating the gene where it is wanted and reducing the risk of gene diffusion to other sites where expression may have deleterious consequences. As discussed above, local transfer of VEGF by, for example, perivascular or intravascular delivery may provide a way of enhancing arterioprotective endothelial functions without stimulating neovascularization at other sites.\(^{41}\)
Cardiac myocytes represent only 30% to 40% of total myocardium, and they cannot replicate to replace cell loss after tissue injury. Targeting myocytes by systemic delivery of a gene and vector is imprecise and inefficient, because the hepatic and pulmonary systems would extract most of the vector before it reached the relevant tissue. Direct injection into the myocardium has been used successfully but alternative, less invasive approaches are preferable. Myocyte transfection via gene transfer to the arterial wall by intravascular techniques is a promising option, which would allow continuous delivery without any exogenous instrumentation (except for the transfection catheter). Another option is the use of coated slow-releasing stents, and an interesting concept to be tested is the use of monocytes as carrier vehicles to the proliferating lesions.

Much of the available data and conclusions regarding the efficacy of cardiovascular gene therapy has derived from monoclonal application. Given the chronic nature, complex etiology, and multifactorial pathogenesis of cardiovascular diseases, targeting a single gene, however attractive a candidate, may prove to be inadequate therapeutically. The same injunction applies to gene cocktails may be the most effective and rational way to achieve optimal therapeutic effects.

References


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