Current Perspective

Perspective in Progress of Cardiovascular Gene Therapy

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Abstract. Recent progress in molecular and cellular biology has developed numerous effective cardiovascular drugs. However, there are still a number of diseases for which no known effective therapy exists, such as peripheral arterial disease, ischemic heart disease, restenosis after angioplasty, vascular bypass graft occlusion, and transplant coronary vasculopathy. Currently, gene therapy is emerging as a potential strategy for the treatment of cardiovascular disease to treat such diseases despite of its limitations. The first human trial in cardiovascular disease was started in 1994 to treat peripheral vascular disease using vascular endothelial growth factor (VEGF). Since then, many different potent angiogenic growth factors have been tested in clinical trials to treat peripheral arterial disease. The results from these clinical trials seem to exceed expectations. Improvement of clinical symptoms in peripheral arterial disease and ischemic heart disease has been reported. In addition, another strategy for combating disease processes, the targeting of transcriptional processes, has been tested in a human trial. Genetically modified vein grafts transfected with decoy against E2F, an essential transcription factor in cell cycle progression, revealed apparent long-term potency in human patients. This review focuses on the future potential of gene therapy for the treatment of cardiovascular disease.

Keywords: cis-element decoy, antisense, angiogenesis, vascular endothelial growth factor, hepatocyte growth factor

I. Introduction

Recent progress in molecular and cellular biology has developed numerous effective cardiovascular drugs. However, there are still number of diseases for which no known effective therapy exists, such as peripheral arterial disease (PAD), ischemic heart disease, restenosis after angioplasty, vascular bypass graft occlusion, and transplant coronary vasculopathy. Currently, gene therapy is emerging as a potential strategy for the treatment of cardiovascular disease despite of its limitations. The advantages of gene therapy, as well as possible limitations, as compared to the classical pharmacological approach are as follows: 1) It has the potential to maintain an optimally high and local concentration of therapeutic genes over time. In the case of therapeutic angiogenesis as discussed later, it may be preferable to deliver a lower dose over a period of several days or more from an actively expressed transgene in the iliac artery, rather than a single or multiple bolus doses of recombinant protein, to avoid side-effects. 2) Regarding economics, which therapy would ultimately cost more to develop, implement, and reimburse, particularly for those indications requiring multiple or even protracted treatment, needs to be considered. 3) The feasibility of a clinical trial of recombinant protein is currently limited by the lack of approved or available quantities of human quality grade of recombinant protein, due in large part to the nearly prohibitive cost of scaling up from research grade to human quality recombinant protein. Indeed, the report to compare the effectiveness of fibroblast growth factor-2 (FGF-2) as protein and as naked plasmid DNA in a porcine model of chronic myocardial ischemia demonstrated that intramyocardial injection of FGF-2 plasmid was more effective than FGF-2 protein in improving regional perfusion and contractility compared to untreated ischemia. In contrast, gene therapy also has the disadvantages such as safety aspects and localized and limited effects.

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Invited article
II. Gene therapy for cardiovascular diseases

1. Gene therapy to treat PAD using therapeutic angiogenesis

Critical limb ischemia that is estimated to develop in 500 to 1000 individuals per million per year is considered as one of the most suitable diseases for gene therapy. In a large proportion of these patients, the anatomical extent and the distribution of arterial occlusive disease make the patients unsuitable for operative or percutaneous revascularization. Consequently, the need for alternative treatment strategies in patients with critical limb ischemia is compelling. Therefore, novel therapeutic modalities are needed to treat these patients. Recently, the efficacy of therapeutic angiogenesis using VEGF (vascular endothelial growth factor) gene transfer has been reported in human patients with critical limb ischemia (1–3), as the strategy for therapeutic angiogenesis using angiogenic growth factors is considered for the treatment of patients with critical limb ischemia. A human clinical trial using the VEGF gene was started in 1994 by Professor JM Isner at Tufts University. An initial trial was performed using a hydrogel catheter with naked VEGF165 plasmid. Although this procedure seems to be effective for stimulating collateral formation in patients with PAD, it is not ideal for treating many patients, as most patients lack an appropriate target vascular lesion for catheter delivery. Thus, his group applied intramuscular injection of naked plasmid encoding the VEGF165 gene (Fig. 1). Exceeding expectation, this clinical trial demonstrated clinical efficacy for treatment of PAD (1–3). Since then, numerous angiogenic growth factors such as VEGF121, VEGF2, and bFGF (basic fibroblast growth factor) has been tested in clinical trials (4, 5). In addition to intramuscular injection of naked plasmid DNA, adenoviral delivery, liposomal delivery of angiogenic growth factors is also utilized in these trials. In addition to the success of the intramuscular injection of VEGF165 and VEGF2 plasmid DNA, local catheter-mediated VEGF165 gene therapy in ischemic lower-limb arteries after percutaneous transluminal angioplasty (PTA) trial was also successful (5). Follow-up DSA revealed increased vascularity in the VEGF-treated groups distal to the gene transfer site and the region of the clinically most severe ischemia (5). Recent report using adenovirus encoding VEGF121 demonstrated the improvement of endothelial dysfunction in response to acetylcholine or nitroglycerine (6). However, a high level of incidence of edema as side effects has been reported in VEGF trial. In case of Fontaine II as intermittent claudication, the recent result from the randomized study of adenoviral VEGF121 gene transfer was not successful (7). The selection of the agent (VEGF121 versus 165), patient population (intermittent claudication versus critical limb ischemia), and outcome measures (peak walking time versus ulcer size) should be considered in the quest for optimal angiogenic strategies that result in the growth of functional blood vessels and improvement in clinical symptoms.

The safety and efficacy of increasing single and repeated doses of intramuscular naked plasmid DNA encoding for FGF1 administered to patients with PAD was also reported (4). A significant reduction in pain and aggregate ulcer size was detected after FGF gene transfer associated with an increased transcutaneous oxygen pressure and ABI (Ankle Pressure Index) (4). We also identified hepatocyte growth factor (HGF) as a novel candidate for therapeutic angiogenesis. We and others reported that HGF stimulated angiogenesis in rabbit ischemic hindlimb model, rat ischemia model, and mouse ischemia models (8). In addition, the angiogenic activity of HGF is more potent than VEGF or bFGF in vitro as well as in vivo. Moreover, transfection of the human HGF gene by naked plasmid DNA or HVJ (hemagglutinating virus of Japan)-liposome method resulted in a significant increase in blood flow. The angiogenic property of transfection of the HGF gene was also proved in a diabetes and high concentration of lipoprotein (a) models. Based upon these findings, we planned a human clinical trial using intramuscular injection of naked human HGF plasmid (0.5 mg × 4 or 8 sites) two times. Currently, HGF gene transfer has been performed in 22 patients with PAD or Buerger disease of Fontaine grade III or IV who had failed conventional therapy. Reduction of pain scale (1 cm in visual analog scale) was observed in 12 of 13 patients (efficacy rate approximately 92%). Increase in ABI to
>0.1 was observed in 11 of 17 patients (efficacy rate 65%), while the reduction of ischemic ulcer size over 25% was observed in 18 of 25 (efficacy rate 72%). Importantly, the serum level of human HGF protein did not change during gene therapy. No acute severe complications or allergic events were observed in any patients. Two-month follow-up studies showed no evidence of the development of neoplasm or hemangioma. It is noteworthy that there was no evidence of edema in the patients transfected with the human HGF gene, in marked contrast to the VEGF trial in which 60% of patients developed moderate or severe edema in a phase I/IIa trial. Currently, the phase III trial in Japan and the phase II in USA to treat PAD are underway. Based upon these properties, it is assumed that first gene therapy drug may be commercially available in 2005.

2. Gene therapy to treat myocardial ischemic disease using therapeutic angiogenesis

A similar idea has been applied to treat coronary artery disease. A human gene therapy trial to treat coronary artery disease using the VEGF165 gene has been started by Professor J.M. Isner (9, 10). His group performed intramuscular injection of naked plasmid encoding VEGF gene into ischemic myocardium through mini-operation. Similar to human trials in PAD, transfection of VEGF gene resulted in a marked increase in blood flow and improved clinical symptoms without apparent toxicity (9). More recently, the results from 13 consecutive patients with chronic stable angina have been reported (10). Although all of them had failed conventional therapy (drugs, PTCA (percutaneous transluminal coronary angioplasty), and/or CABG (coronary artery bypass grafting)), reduction in the size of the defects documented by serial single-photon emission CT-sestamibi imaging was observed after direct myocardial injection of phVEGF165 via a minithoracotomy (10). These data clearly suggest that phVEGF165 gene therapy may successfully rescue foci of hibernating myocardium. In addition, the recent report summarized the anesthetic management of 30 patients with class 3 or 4 angina, enrolled in a Phase I clinical trial of direct myocardial gene transfer of naked DNA-encoding VEGF165, as sole therapy for refractory angina. Twenty-nine of 30 patients experienced reduced angina and sublingual nitroglycerin consumption (11). Even at 1-year follow-up, the average number of angina episodes per week and average number of nitroglycerin tablets used per week significantly improved at all measured time points after gene transfer (12). Following this success, a phase I study using intramuscular injection of adenoviral vector of VEGF121 gene demonstrated clinical safety (13). It is noteworthy that no evidence of systemic or cardiac-related adverse events related to vector administration was observed up to 6 months after therapy (14). Intracoronary gene transfer of VEGF165 resulted in a significant increase in myocardial perfusion, although no differences in clinical restenosis rate or minimal lumen diameter were present after the 6-month follow-up (15). More recently, intra-coronary infusion of adenovirus encoding FGF gene was performed in a multi-center trial as phase I/IIa. The report documented that intra-coronary infusion of FGF gene improved cardiac dysfunction without severe toxicity (16). In addition, the report to treat 52 patients with stable angina and reversible ischemia documented that FGF4 adenoviral injection resulted in a significant reduction of ischemic defect size (17). Currently, the phase IIb/III trials using adenoviral delivery of FGF4 are now underway. In addition to these angiogenic growth factors, over-expression of HGF was also reported to stimulate angiogenesis and collateral formation in a rat and canine myocardial infarction model (18, 19). More recently, an anti-fibrotic action of HGF has been identified, as HGF inhibited collagen synthesis through tumor growth factor (TGF)-β and stimulated collagen degradation through up-regulation of MMP-1 and uPA. Thus, HGF may also provide a new therapeutic strategy to treat fibrotic cardiovascular disease; that is, cardiomyopathy. Our group has also applied to start a human gene therapy protocol using intra-cardiac-muscular injection of HGF plasmid DNA through surgical operation. Overall, the treatment for coronary artery disease may also be curable using therapeutic angiogenesis by gene therapy.

3. Gene therapy for restenosis after angioplasty

Another important disease potentially amenable to gene therapy in cardiovascular disease is restenosis after angioplasty, since the long term effectiveness of this procedure is limited by the development of restenosis in over 40% of patients. Balloon angioplasty is one of the major therapeutic approaches to coronary artery stenosis. However, restenosis occurs in 30% to 40% of patients after angioplasty. Intimal hyperplasia develops in large part as a result of vascular smooth muscle cell (VSMC) proliferation and migration induced by a complex interaction of multiple growth factors that are activated by vascular “injury”. The process of VSMC proliferation is dependent on the coordinated activation of a series of cell cycle regulatory genes that results in mitosis. Therefore, inhibition of the cell cycle using non-phosphorylated Rb (retinoblastoma) gene or anti-oncogenes such as p53 and p21 has been reported in several animal models. Recently,
over-expression of inducible nitric oxide synthase gene has been tested in human subjects, although the results are not yet published.

Alternatively, it has been hypothesized that rapid regeneration of endothelial cells without replication of VSMC may also modulate vascular growth, because multiple anti-proliferative endothelium-derived substances (PGI₂, NO, CNP) are secreted from endothelial cells. Isner et al. reported a significant inhibition of neointimal formation by acceleration of endothelial cells replication by VEGF gene transfer (20). Based upon this finding, a human trial using VEGF165 gene by hydrogel catheter delivery of naked VEGF165 plasmid DNA has been started for restenosis after angioplasty in peripheral artery (20). Although the final results have not yet been reported, the preliminary results documented the successful inhibition of restenosis after angioplasty. A similar trial using VEGF165 gene has been started in Finland. In this trial, VEGF gene was transfected by cationic liposome or adenovirus with a catheter into the coronary artery (21). A recent report demonstrated the clinical safety of VEGF gene transfer with cationic liposome or adenovirus (21). Although gene transfer with VEGF using adenovirus during PTCA and stenting shows that intracoronary gene transfer can be performed safely (no major gene transfer-related adverse effects were detected), no differences in clinical restenosis rate or minimal lumen diameter were present after the 6-month follow-up (15).

Nevertheless, a significant increase was detected in myocardial perfusion in the VEGF-treated patients (15). In addition, we also reported preclinical experiments in which over-expression of HGF gene in balloon-injured arteries could accelerate re-endothelialization, thereby attenuating intimal hyperplasia, associated with the improvement of endothelial dysfunction (22). Further studies are necessary to clarify the utility of gene therapy to treat restenosis after angioplasty.

III. Gene therapy for cardiovascular disease using oligonucleotide-based strategy

1. Antisense or ribozyme-based gene therapy

Recent progress in molecular biology has provided new techniques to inhibit target gene expression. Especially, the application of DNA technology such as antisense strategy to regulate the transcription of disease-related genes in vivo has important therapeutic potential. Antisense oligodeoxynucleotides (ODN) are widely used as inhibitors of specific gene expression because they offer the exciting possibility of blocking the expression of a particular gene without any change in function of other genes (see Fig. 2). Therefore, antisense ODN are useful tools in the study of gene function and may be potential therapeutic agents. First, the effectiveness of antisense ODN against a proto-oncogene, c-myb, was reported for the treatment of restenosis (23). Accordingly, inhibition of other proto-oncogenes
such as c-myc by antisense ODN was also reported to inhibit neointimal formation in several animal models. Recently, the results from a phase II trial using antisense c-myc to treat restenosis has been reported (24). Treatment with 10 mg of phosphorothioate-modified ODN directed against c-myc does not reduce neointimal volume obstruction or the angiographic restenosis rate (24). However, this trial utilized intra-coronary infusion of antisense c-myc ODN without any vectors, and several issues such as low transfection efficiency may limit the efficacy of this strategy.

On the hand, the process of VSMC proliferation is dependent on the coordinated activation of a series of cell cycle regulatory genes that results in mitosis. Our previous data revealed that a single administration of antisense ODN against PCNA (proliferative cell nuclear antigen)/cdc2 kinase genes or cyclin B1/cdc2 genes significantly inhibited the extent of neointimal formation after transfection (25). Similarly, transfection of antisense ODN against PCNA and cdc2 kinase resulted in the inhibition of hyperplasia at 2 weeks after transfection in a vein graft model. Moreover, the prevention of neointimal formation in the balloon injury model by the cell cycle inhibition strategy was sustained long term over the period of antisense survival. This may relate to the vascular remodeling induced by the inhibition of cell cycle progression. Indeed, administration of antisense PCNA and cdc2 kinase ODN into a vein graft model improved the resistance to diet-induced atherogenesis. In addition to the prevention of restenosis after vein grafting, the inhibition of hyperplasia in transplanted hearts has also been reported by Suzuki et al. Transfection of antisense cdk2 kinase ODN resulted in significant inhibition of VSMC growth in the transplanted heart (26). The first antisense drug appeared on the market in the USA at the end of 1999 as a novel drug to treat cytomegaloviral-mediated retinopathy.

Another strategy for combating disease processes by targeting to the transcriptional process is the use of ribozymes, a unique class of RNA molecules that not only store information but also process catalytic activity. Ribozymes are known to catalytically cleave specific target RNA leading to degradation, whereas antisense ODN inhibit translation by binding to mRNA sequences on a stoichiometric basis. Theoretically, ribozymes are more effective to inhibit target gene expression. However, no clinical trial is underway in the cardiovascular field.

2. Decoy-based gene therapy

More recently, we have found a novel molecular strategy in which synthetic double-stranded (ds) DNA with high affinity for a target transcription factor may be introduced into target cells as a “decoy” cis element to bind the transcription factor and alter gene transcription (27). Transfection of ds ODN corresponding to the cis sequence will result in the attenuation of the authentic cis-trans interaction, leading to the removal of trans-factors from the endogenous cis-element, with subsequent modulation of gene expression. Therefore, the decoy approach may also enable us to treat diseases by modulation of endogenous transcriptional regulation. Recently, several studies have demonstrated application of the “decoy” ODN strategy as in vivo gene therapy (27) and provide evidence of in vivo application of this novel molecular approach as a therapeutic strategy against cardiovascular disease.

As discussed above, the process of VSMC proliferation is dependent on the coordinated activation of a series of cell cycle regulatory genes, which results in mitosis. A critical element of cell cycle progression regulation involves the complex formed by E2F, cyclin A, and cdk2. The dissociation of the transcription factor E2F from the retinoblastoma gene product is proposed to play a pivotal role in the regulation of cell proliferation by inducing coordinated transactivation of genes involved in cell cycle regulation including c-myc, c-myc, cdc2, PCNA, and thymidine kinase. Accordingly, we hypothesized that transfection of VSMC with a sufficient quantity of decoy ODN containing the E2F cis element (consensus sequence “TTTTCGGCGC”) would effectively bind E2F, prevent it from transactivating the gene expression of essential cell cycle regulatory proteins and thereby inhibit VSMC proliferation and neointimal formation. Transfection of E2F decoy ODN into rat balloon-injured carotid arteries and porcine coronary arteries resulted in almost complete inhibition of neointimal formation after balloon injury (28). Based on these results, we started a clinical trial using hydrogel catheter delivery of E2F decoy to treat restenosis after angioplasty in April 2000. As of January 2004, we have treated five patients with E2F decoy ODN. We did not observe any side effects up to 6 months, although the clinical outcome has not yet been evaluated.

In addition, in 1996, clinical application of “decoy” against E2F by Dr. V.J. Dzau at Harvard University was also approved by the FDA to treat neointimal hyperplasia in vein bypass grafts, which results in failure in up to 50% of grafts within a period of 10 years. A proof-of-concept study, the Project in Ex-Vivo Vein Graft Engineering Via Transfection (PREVENT I) study, was the first clinical trial using genetic engineering techniques to inhibit cell-cycle activation in vein grafts (29). This prospective, randomized, controlled trial demonstrated the safety and biologic efficacy of
intraoperative transfection of human bypass vein grafts with E2F decoy oligonucleotides in a high-risk human patient population with peripheral arterial occlusion. They demonstrated successful inhibition of graft occlusion, accompanied by selective inhibition of PCNA and c-myc expression (29). More recently, similar results were obtained in PREVENT II, a randomized, double-blind, placebo-controlled trial investigating the safety and feasibility of E2F decoy oligonucleotides in preventing autologous vein graft failure after coronary artery bypass surgery. The interim results confirmed the safety and feasibility of using this product. Analysis of the secondary end points using quantitative coronary angiography and 3-dimensional intravascular ultrasound demonstrated increased patency and positive vascular remodeling (inhibition of neointimal size and volume) in the treated group at 12 months. Patients examined at follow-up were found to have, on average, a 40% reduction in critical stenosis. Further assessment of this encouraging therapeutic approach should be completed by 2005 in adequately powered phase 3 studies in coronary and peripheral vessel disease to determine definitively the extent and duration of clinical benefit. Since E2F has been postulated to play an important role in the pathogenesis of numerous diseases, for example, vasculopathy after transplantation, the development of the E2F decoy strategy may provide a useful therapeutic tool for treating these proliferative diseases.

On the other hand, the transcription factor NFκB also plays a pivotal role in the coordinated transactivation of cytokine and adhesion molecule genes whose activation has been postulated to be involved in numerous diseases, such as myocardial infarction. Numerous cytokines including interleukin (IL)-1, 2, 6, 8 and TNF-α, to name a few, regulate this process. However, gene regulation of many cytokines is relatively simple because the transcription factor NFκB has been reported to up-regulate these cytokines. Interestingly, adhesion molecules such as vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) are also known to be up-regulated by NFκB. Importantly, increased NFκB binding activity has been confirmed in balloon-injured blood vessels. Our recent study provided the first evidence of the feasibility of a decoy strategy against NFκB in treating restenosis (Fig. 3) (30). Transfection of NFκB decoy ODN into balloon-injured carotid artery or porcine

Fig. 3. Mechanisms of NFκB decoy ODN. ELAM = endothelial-leukocyte adhesion molecule, 1κB = inhibitor of NFκB.
coronary artery markedly reduced neointimal formation, whereas no difference was observed between scrambled decoy ODN-treated and untransfected blood vessels. Based upon the therapeutic efficacy of this strategy, we obtained permission for a second clinical trial using the decoy strategy to treat restenosis from 2001. In addition, the inhibition of VSMC replication was confirmed by the observation that transfection of NFκB decoy ODN inhibited the progression of vasculopathy in cardiac transplantation models. Blockade of NFκB is also effective to treat reperfusion myocardial injury. Transfection of NFκB decoy ODN into rat coronary artery prior to or after LAD occlusion markedly reduced the area of damaged myocytes at 24 h into rat coronary artery prior to or after LAD occlusion.

IV. Perspectives in gene therapy

Gene therapy in the field of cardiovascular disease would be useful for the treatment of many diseases, including PAD, myocardial infarction, restenosis after angioplasty, and rejection in heart transplantation. The first federally approved human gene therapy protocol started on September 14, 1990 for ADA (adenosine deaminase) deficiency patients. Over ten years since the commencement of the first trial, over 4000 patients have been treated by gene therapy. The objectives are generally to evaluate 1) the in vivo efficacy of the gene transfer method, 2) the safety of the gene transfer method, and 3) the possible therapeutic efficacy. Although there are still many unresolved issues in the clinical application of gene therapy, gene therapy for cardiovascular disease now appears to be not far from reality and it is time to take a hard look at practical issues that will determine the real clinical potential. These include 1) further innovations in gene transfer methods, 2) well-defined disease targets, 3) cell-specific targeting strategies, and 4) effective and safe delivery systems.

References


