

Gene therapy for in-stent restenosis: Targets and delivery system

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Although the rate of target lesion revascularization has been reduced by the advent of the drug-eluting stent, the incidence of in-stent restenosis remains unacceptably high. Gene therapeutic strategies, including catheter-based gene delivery and gene-eluting stents, offer novel treatment methods to promote re-endothelialization, and inhibit inflammation, neointimal hyperplasia and late stent thrombosis. The translation of gene therapy into clinical application must be safe and requires an effective,

Despite the increased use of drug-eluting stents (DES), the incidence of in-stent restenosis (ISR) requiring target vessel revascularization remains unacceptably high (1,2). The incidence rate of ISR requiring target vessel revascularization (ie, 'DES failure') in the United States alone was conservatively estimated to be 10%, and may be more common in patients with diabetes mellitus (3). Although the pathophysiology of DES failure is complex, histopathological changes of in-stent neointima following directional atherectomy at the time of reintervention have been shown to be remarkably similar between bare-metal stents (BMS) and DES, suggesting that a process mediated by the endothelial injury, inflammation, proliferation and migration of vascular smooth muscle cells (VSMCs), and thrombosis plays the same important role in the progress of ISR of DES (4,5). In addition, anti-proliferative drugs in DES, primarily aimed at preventing VSMC proliferation (which is central to the pathogenesis of ISR), also perturb endothelial recovery (6), which consequently increases the risk for subacute in-stent thrombosis and results in late stent thrombosis (ST) (7). Furthermore, polymer coatings on DES induce a distinct inflammatory reaction compared with bare metal surfaces (8). These results raise serious questions regarding the long-term durability and efficacy of DES, thereby resulting in an urgent need to develop new therapies to prevent restenosis. The present review focuses on the progress made to date in the use of gene therapy, including catheter-based gene delivery and gene-eluting stents (GES), to prevent ISR. We will also discuss the current and promising application of magnetic targeting gene delivery systems for antirestenosis therapy.

EVOLUTION OF ANTIRESTENOSIS THERAPY

In the past several decades, percutaneous transluminal coronary angioplasties have revolutionized the treatment of atherosclerosis-related cardiovascular disease. At the same time, tremendous efforts have also been made to reduce the incidence of restenosis after percutaneous transluminal coronary angioplasties (9). During the time in which 'plain old balloon angioplasty' was prevalent, rates of acute and chronic vessel occlusion were higher (30% to 56%), secondary to acute/chronic recoil and constrictive remodelling (10). These problems led to the development of a second revolutionary treatment, BMS, which were first implanted in 1986 (11). Although this 'bailout'

site-specific delivery system as well as the ability to provide sustained transgene expression. The progress of magnetic nanotechnology and genetic engineering of human stem cells can provide such elements. In the present review, the authors discuss the evolution of antirestenosis therapy, underlying mechanisms of restenosis and the applications of gene therapy to prevent in-stent restenosis. Current gene delivery methods, including gene vectors and delivery strategies, are critically reviewed.

Key Words: *Gene therapy; Intimal hyperplasia; Nanotechnology; Re-endothelialization; Restenosis; Stem cell*

scaffold significantly reduced the issue of acute and chronic recoil, its application was ultimately hampered by the risk for subacute thrombotic coronary artery occlusion and neointimal hyperplasia (12). The restenosis rates with BMS were reported to be between 16% and 44%, with higher rates of stenosis attributable to several risk factors, particularly long lesion length and small vessel calibre (1,13).

Considerable progress to reduce the incidence of restenosis was made by the advent of DES, which are composed of a metallic stent, a polymer-based drug delivery platform and a pharmacological agent (typically an immunosuppressant and/or antiproliferative compounds, including sirolimus and paclitaxel [PTX]) (14). Several classic studies unequivocally demonstrated that DES markedly reduced rates of ISR to 5% to 8% (15,16). However, long-term follow-up studies have raised concerns about the long-term safety of DES, although DES definitely improves short- to midterm ISR (17). The application of DES comes with an increased risk for ST because the pathophysiology of ISR and ST appear to be diametrically opposed. Briefly, ISR is related to an overzealous response of the vessel to injury, whereas ST occurs due to impaired or delayed endothelium healing, resulting in acute and subacute thrombotic events (14). There is an urgent need, therefore, to develop novel and efficacious methods to deal with the problems observed in current antirestenosis therapy (Figure 1). Recently, Deuse et al (18) reported the identification of pyruvate dehydrogenase kinase isoform 2 as a key regulatory protein in the development of myointimal hyperplasia in human arteries for its regulated effect on hyperpolarization of mitochondrial membrane potential and resistance to apoptosis in smooth muscle cells (SMCs) during ISR. Normally, proliferation and apoptosis are very low and balanced in healthy vessels. However, vessel injury induces inflammatory response and temporary mitochondrial membrane potential hyperpolarization, which results in the temporarily acquired apoptosis resistance in SMCs and promotes myointima formation. Dichloroacetate, a pharmacological pyruvate dehydrogenase kinase isoform 2 blockade, counteracts temporarily acquired apoptosis resistance and reduces myointima formation in injured coronary arteries of human and various animal models (18). Most importantly, dichloroacetate did not affect vessel re-endothelialization, suggesting a novel strategy for the prevention of proliferative vascular diseases, especially ISR. Moreover,

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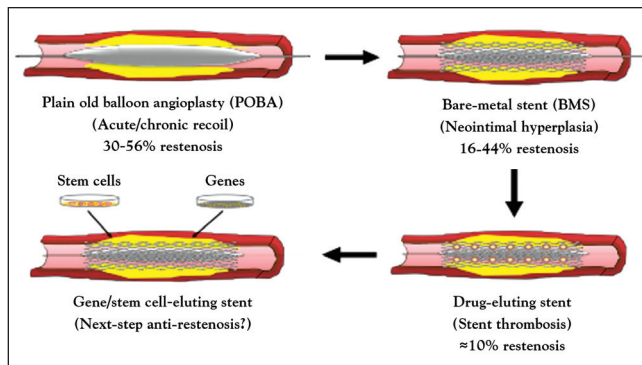


Figure 1) Schematic representation of the evolution of antirestenosis therapy and potential therapeutic strategies for antirestenosis.

magnetic nanotechnology and genetic engineering of human stem cells have also emerged as exciting alternatives to eliminate the underlying mechanisms of ISR, suggesting their possible roles as the next generation of antirestenosis therapy.

THERAPEUTIC GENE TARGETS

Re-endothelialization

Stent implantation for coronary artery disease causes a marked vascular injury, inducing de-endothelialization, which is associated with thrombus formation and abnormal responses to endothelium-dependent agonists (19). In recent years, several studies have focused on the use of vascular gene therapy targeting multiple pathways to promote re-endothelialization (20-26). The potential of vascular endothelial growth factors (VEGFs), a group of potent endothelial mitogens, to treat postangioplasty restenosis was first investigated in animal models. Several VEGF family members, including VEGF165, VEGF-2, VEGF-C and VEGF-D, have been found to inhibit neointimal thickening, reduce thrombogenicity and restore the endothelium-dependent vasomotor reactivity (27-29). Yang et al (23) developed a stent coated with bilayered poly-lactide-coglycolide (PLGA) nanoparticles (NPs) containing VEGF plasmid in the outer layer and PTX in the inner core and found that it significantly promotes early endothelium healing while inhibiting SMC proliferation through sequential release of the VEGF gene and PTX (23).

Another interesting target is nitric oxide (NO), which is mainly synthesized by NO synthase (NOS). Most studies have focused on two main types of NOS, inducible NOS and endothelial NOS (eNOS), to reduce neointimal hyperplasia and accelerate re-endothelialization (30-33). Both catheter- and stent-based delivery techniques have verified eNOS and inducible NOS as potential therapeutic genes for accelerating re-endothelialization and reducing neointimal hyperplasia (Tables 1 and 2) (31-33). Sharif et al (31) found that eNOS gene delivery can result in significantly enhanced re-endothelialization and decreased neointimal formation using an adenovirus GES. The same laboratory reported that nonviral, liposome-based gene delivery of eNOS to the blood vessel wall in vivo results in enhanced endothelialization as well as prolonged and localized eNOS expression in the blood vessel wall in a hypercholesterolemic rabbit model (22). Cyclooxygenase-1 (COX-1) is the rate-limiting component in the synthesis of prostacyclin (PGI₂), a potent vasodilator and antithrombotic molecule. In balloon-injured, atherosclerotic porcine and hypercholesterolemic rabbit models, COX-1 gene transduction induced an early increase in the production of PGI₂, prostaglandin E₂ and prostaglandin E₁, resulting in long-lasting vasodilation (34).

The metabolic syndrome is defined as a cluster of numerous cardiovascular risk factors that encompass dyslipidemia and hypertension. Patients with the metabolic syndrome are more prone to developing ISR (35). Lectin-like oxidized low-density lipoprotein

receptor-1 (LOX-1) is a membrane protein that can bind and internalize oxidized low-density lipoprotein and advanced glycation end products, which further promote endothelial injury after percutaneous coronary interventions (PCI). Targeting the LOX-1 gene with gene silencer pyrrole-imidazole polyamide significantly decreased the expression of LOX-1 and promoted re-endothelialization, thus inhibiting neointimal hyperplasia after arterial injury (24). Gene transfer of PGI₂ synthase has also been shown to accelerate re-endothelialization and prevent neointimal formation in balloon-injured arteries. In a rabbit model, PGI₂ synthase gene delivery by the lipotransfection method via dispatch catheter can accelerate re-endothelialization and attenuate neointimal formation (36). Douglas et al (20) found that endothelial-specific overexpression of GTP-cyclohydrolase-1 can enhance endothelial cell function and increases NO production, thus promoting re-endothelialization after stent deployment.

Antineointimal hyperplasia

Following injury of coronary arteries during interventional procedures, including balloon angioplasty and/or stent placement, medial SMCs change their phenotype from contractile to synthetic, proliferative and migrative in nature, initiating the process of neointimal hyperplasia (37). Therefore, proliferation and migration of SMCs are crucial targets for the inhibition of intimal hyperplasia and restenosis. A number of studies have shown that transfer of cytotoxic antiproliferative genes, including thymidine kinase, cytosine deaminase and Fas ligand, which selectively destroy SMCs as they enter the S phase of the cell cycle, are able to significantly inhibit neointimal hyperplasia in balloon-injured vessels (38-40). In addition, the transfection of antisense oligodeoxynucleotides (ODNs) targeting DNA binding proteins and transcription factors – including CDC2 and cyclin G1, which encode for regulatory proteins that are involved in the cell cycle – have also been shown to inhibit neointimal formation (41,42). MicroRNA, a 21 to 24 nucleotide RNA molecule, inhibits their messenger RNA target by binding to complementary ‘seed’ sequences, leading to post-transcriptional repression or target messenger RNA degradation. Several microRNAs have been found to significantly reduce injury-induced neointima formation by inhibiting VSMC proliferation (43,44). Merlet et al (43) found an upregulation of miR-424/322 after vascular injury. Overexpression of miR-424/322 in injured rat carotid arteries using an adenovirus has been demonstrated to inhibit restenosis.

Apart from the antiproliferative approach, inhibition of the migration of SMCs can reduce neointimal hyperplasia. Extracellular matrix degradation has been shown to play a crucial role in SMC migration, which is mainly regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) (45). Although early studies using marimastat, a broad-spectrum MMP inhibitor, showed significantly reduced neointimal formation in cultured human saphenous vein graft segments, the potential of marimastat as an antimetastatic agent was halted at phase III due to a lack of efficacy and dose-limiting toxicity (46). The potency of TIMP-1 as a therapeutic gene for antineointimal hyperplasia has been verified in various animal models (47,48). Johnson et al (48) found that stent-based delivery of TIMP-3 adenovirus inhibits neointimal formation in porcine coronary arteries (48). Downregulation of platelet-derived growth factor- β receptor expression and upregulation of plasminogen activator inhibitor-1 and light-type caldesmon, a potent cytostatic and antiangiogenic protein, using a gene targeting and adenoviral gene transfer approach, has also been found to inhibit neointima formation (49). Lompré et al (50) investigated the functional effects of adeno-associated virus (AAV) 2/5-mediated sarco/endoplasmic reticulum Ca²⁺ATPase isoform 2a (SERCA2a) – a human gene known to inhibit VSMC proliferation – in a rat model of carotid artery balloon injury and found that AAV2/5-mediated SERCA2a gene transfection significantly decreases the neointimal hyperplasia without inducing obvious inflammation in vivo.

Activation of the tyrosine kinase family JAK and subsequent activation of signal transducer and activator of transcription (STAT)

TABLE 1
Summary of major studies using nonviral vectors for antirestenosis gene therapy

| Author (reference) | Vector(s) | Gene(s) | Model/patients | Main outcome(s) |
|------------------------|---|----------------------------|---|--|
| Taniyama et al (67) | Naked plasmid DNA | <i>p53</i> | Rat carotid artery after balloon injury | Transgene expression of naked p53 plasmid DNA using ultrasound transfection method decreased the intimal-to-medial area ratio |
| Van Belle et al (69) | Naked plasmid DNA | <i>VEGF 165</i> | Rabbit artery stent restenosis model | Transgene expression of naked VEGF165 plasmid DNA decreased mural thrombus and intimal thickening |
| Fuchs et al (64) | Naked plasmid DNA | C-type natriuretic peptide | Porcine restenosis model | Transgene expression of naked C-type natriuretic peptide plasmid DNA resulted in significant inhibition of neointimal formation without compromising endothelial repair |
| Zhang et al (62) | Naked plasmid DNA and anti-DNA antibody-immobilized stent | <i>iNOS</i> | Porcine coronary stent model | Tethering of pcDNA3.1-iNOS on the stent showed long-term therapeutic effects for antirestenosis |
| Chang et al (99) | Protamine sulfate plasmid DNA | Hepatocyte growth factor | Rat carotid artery injury model | The protamine sulfate/hepatocyte growth factor-pDNA multilayer coated stent can reduce in-stent restenosis in vivo |
| Brito et al (33) | Liposomes plasmid DNA | <i>eNOS</i> | Rabbit artery stent restenosis model | Efficient transfection of eNOS locally in the arterial lumen suppressed SMC proliferation and promoted re-endothelialization of the artery showing a significant reduction of restenosis |
| Deiner et al (100) | Liposomes plasmid DNA | <i>VEGF-165</i> | Balloon-injured porcine coronary arteries model | Local delivery of <i>VEGF-165</i> gene into the outer compartments of balloon-injured porcine coronary arteries reduced lumen area loss due to distinct positive remodelling |
| Yang et al (63) | PLGA/NPs plasmid DNA | Antisense for <i>MCP-1</i> | Rabbit carotid artery injury model | Local infusion of anti-MCP-1 NPs significantly lowered the intimal hyperplasia |
| Walter et al (65) | Phosphorylcholine polymer plasmid DNA | <i>VEGF-2</i> | Rabbit artery stent restenosis model | Transgene expression of VEGF-2 in the vessel wall improved functional recovery of stented segments |
| Hedman et al (66) | Liposomes plasmid DNA | <i>VEGF</i> | Patients with coronary artery disease | Transgene expression of VEGF cannot improve the ISR in human after the 6-month follow-up |
| Egashira et al (56) | ODN | Antisense NF-κB ODN | Patients with coronary artery disease | Transfection of NF-κB decoy ODN can obviously suppress the ISR in human |
| Matsushita et al (101) | ODN | Antisense p53 ODN | Rat intact carotid artery model | Loss of p53 by antisense p53 ODN resulted in an abnormal VSMC growth in vitro and in vivo |
| Nikol et al (68) | Liposomes plasmid DNA | Cecropin A | Porcine arterial injury model | Transfection of cecropins A prevented restenosis by limiting SMC proliferation In vivo |
| Chapman et al (102) | Liposomes plasmid DNA | Luciferase | Mongrel dogs model | The first study to show the efficiency of nonviral gene transfection using percutaneous catheter |

eNOS Endothelial nitric oxide synthase (*NOS*); *iNOS* Inducible *NOS*; *ISR* In-stent restenosis; *MCP-1* Monocyte chemoattractant protein-1; *NPs* Nanoparticles; *ODNs* Oligodeoxynucleotides; *PLGA* Poly-lactide-coglycolide; *SMC* Smooth muscle cell; *VEGF* Vascular endothelial growth factor; *VSMC* Vascular smooth muscle cells

proteins are key events in signal transduction of different cytokines and growth factors. These proteins regulate different biological processes including SMC proliferation (51). Suppressor of cytokine signalling-3 (SOCS3) protein decreases the JAK/STAT signalling by blocking the JAK tyrosine kinase activity and the activation of STAT factors, suggesting that it is a novel antineointimal hyperplasia target. Recently, our laboratory has found a significant reduction in SOCS3 expression in the neointimal lesion after balloon angioplasty in coronary arteries of atherosclerotic swine (52). In addition, our laboratory demonstrated that hypermethylation of the SOCS3 gene in SMCs could be an underlying mechanism of intimal hyperplasia and restenosis, suggesting that inhibition of SOCS3 promoter methylation is a potential anti-neointimal hyperplasia target (53).

Anti-inflammation

Inflammation is a protective attempt by the organism to remove the injurious stimuli. However, stent implantation, including BMS and polymer-coated DES implantation, induces acute and chronic inflammation that stimulates accumulation of inflammatory cells, such as macrophages and T-lymphocytes, and inflammatory cytokine secretion (8). Inhibition of inflammatory gene expression may have the therapeutic effect of reducing neointimal hyperplasia. Transforming growth factor-beta (TGF-β) is a crucial inflammatory cytokine that controls immunity/inflammation and cell proliferation. Delivery of the gene silencer pyrrole-imidazole polyamide, which targets the TGF-β1 promoter, can suppress neointimal hyperplasia after

arterial injury by downregulating TGF-β1 expression and inhibiting restenosis (25). Monocyte chemoattractant protein-1 (MCP-1) is a proinflammatory chemokine specific for monocytes and is shown to be present at increased levels after vascular injury. The peptide from the C-terminal domain of MCP-1 (ingramon) has been shown to inhibit monocyte migration and possess anti-inflammatory activity while at the same time inhibiting the postangioplasty restenosis in animal models (54). In rabbit and monkey models, the same laboratory found that the local delivery of the C-terminal domain of MCP-1 gene (7ND) by a GES system attenuates in-stent stenosis (55). In cynomolgus monkeys, a catheter-based anti-MCP-1 gene delivery system has also been shown to attenuate in-stent neointima formation (55).

As an essential transcriptional factor for inflammation, nuclear factor-kappa B (NF-κB) has been found to play a pivotal role for restenosis after PCI. In an open-label phase I/IIa clinical trial, Egashira et al (56) found that NF-κB decoy ODN transfection can suppress the inflammatory response and prevent restenosis. CREB-binding protein is a powerful transcriptional coactivator that regulates inflammation, cell proliferation and apoptosis in vascular endothelial and SMCs. Knockdown of CREB-binding protein using small interfering RNA demonstrated a potent inhibitory effect on balloon injury-induced NF-κB acetylation and attenuated neointimal formation in balloon-injured rat carotid artery (57). Activator protein-1 is another crucial transcription factor regulating gene expression in response to a variety of proinflammatory stimuli. Using

TABLE 2
Summary of major studies using viral vector for antirestenosis gene therapy

| Author (reference) | Vector(s) | Gene(s) | Model/patients | Main outcome(s) |
|---------------------------|--|---|---|---|
| Li et al (44) | Adenovirus | miR-663 | Mice carotid artery injury model | Adeno-miR-663 markedly suppressed the neointimal lesion formation in mice |
| Merlet et al (43) | Adenovirus | miR-424/322 | Rat carotid artery injury model | Adeno-miR-424/322 markedly suppressed the neointimal lesion formation in rats |
| Chorny et al (77) | Adenovirus | Magnetic nanoparticles | Rat carotid artery injury model | Magnetically targeted adenovirus-loaded magnetic nanoparticles represent a novel delivery system for efficient, vascular gene transfer in vivo |
| Wu et al (80) | Adenovirus | Dominant-negative <i>Skp2</i> | Rat carotid artery injury model | Knockdown of <i>Skp2</i> inhibited VSMC proliferation, and the subsequent neointimal thickening in rat arteries |
| Fishbein et al (103) | PABT/PEI(PDT)/HL-tethered adenoviral vectors | <i>iNOS</i> | Rat carotid artery stent model | Rat carotid stent delivery of <i>iNOS</i> resulted in significant inhibition of restenosis in rats |
| Johnson et al (48) | Adenovirus | <i>TIMP-3</i> | Porcine coronary stent model | Adenovirus-coated <i>TIMP-3</i> stent significantly inhibited the in-stent neointimal formation in pigs |
| Hedman et al (66) | Adenovirus | <i>VEGF</i> | Patients with coronary artery disease | Myocardial perfusion showed a significant improvement in the <i>VEGF</i> -adenovirus-treated patients after the six-month follow-up |
| Sinnaeve et al (104) | Adenovirus | <i>PKG</i> mutant | Porcine coronary stent model | Expression of a constitutively active <i>PKG</i> reduces neointimal formation after balloon injury in rats and reduces coronary in-stent restenosis in pigs |
| Liu et al (34) | Adenovirus | <i>COX-1</i> | Rabbits carotid artery balloon injury model | Local gene transduction of <i>COX-1</i> increases blood flow in injured atherosclerotic rabbit arteries |
| Lompré et al (50) | AAV2.5 | Sarco/endoplasmic reticulum Ca^{2+} ATPase isoform 2a | Rat carotid artery balloon injury model | AAV2.5 vector can be considered as a promising safe and effective vector for antirestenosis gene therapy in vivo |
| Sharif et al (79) | AAV2 | DNase-resistant particles | Rabbits iliac artery stent model | AAV2-coated stents can be used to deliver genes to the blood vessel wall for up to 28 days in vivo |
| Pankajakshan et al (82) | AAV2/9 | <i>SM22α</i> promoter | Swine coronary and peripheral arteries | AAV2/9 vector can be considered as a promising safe and effective vector for antirestenosis gene therapy in vivo |
| Ramirez Correa et al (78) | Recombinant AAV | <i>TIMP-1</i> | Rat carotid injury arteries | Local AAV- <i>TIMP-1</i> gene transfer represents an efficient strategy to prevent restenosis in rats |
| Squadrito et al (105) | Recombinant AAV | NF- κ B inhibitory protein I κ B α | Mice carotid artery injury model | Recombinant AAV-mediated gene transfer of I κ B α inhibited the restenosis in mice |
| Yang et al (57) | Lentivirus | <i>CBP</i> | Rat carotid injury arteries | Lentivirus-mediated <i>CBP</i> silencing may represent a novel therapeutic approach for the prevention of restenosis after vascular interventions in rats |
| Bonta et al (76) | Lentivirus | <i>Nurr1</i> | Rat carotid injury arteries | Lentivirus-mediated nuclear receptor <i>Nurr1</i> is an attractive novel target for (local) intervention in restenosis in rats |

AAV Adeno-associated virus; CBP CREB-binding protein; COX Cyclooxygenase; *iNOS* Inducible nitric oxide synthase; *Nurr1* Nuclear receptor-related 1; *PKG* Protein kinase G; *TIMP* Tissue inhibitor of metalloproteinase; *VEGF* Vascular endothelial growth factor; *VSMC* Vascular smooth muscle cell

decoy ODN transfection against the activator protein-1 binding site has recently been shown to effectively increase re-endothelialization and inhibit neointimal proliferation (21).

Antithrombosis

Stent implantation-induced thrombosis is a classical example of device-induced, platelet-mediated arterial thrombosis (58). In a porcine model of coronary restenosis, anti-CD34 antibody-coated sirolimus-eluting stents were associated with greater endothelialization, thus inhibiting platelet activation. However, a clear clinical benefit of stents coated with this technology has not been shown (58,59). ADP, a key agonist required to trigger platelet aggregation, has received much attention as a potential antithrombosis therapeutic target. Takemoto et al (60) found that transfection of the human pE-NTPDase gene, encoding an enzyme that rapidly hydrolyzes ATP and ADP to AMP via cationic gelatin-coated stents, inhibits subacute in-stent thrombosis and suppresses neointimal hyperplasia and inflammation without antiplatelet drugs. More established antithrombotic genes, including PGI₂ synthase, COX-1 and tissue factor pathway inhibitor, have been investigated in recent years for their potential in postangioplasty intervention, and have

demonstrated successful suppression of thrombosis and/or restenosis in various animal models (34,36,58,61).

GENE DELIVERY SYSTEMS

Gene vectors

Viral vector and nonviral vector are the classic gene delivery vectors, both of which have the ability to introduce therapeutic genes into vascular tissue (6). For greater safety and less immunogenicity, nonviral vectors have held more promise for clinical application than viral vectors. However, the complex process of migration through the vascular endothelium and the extracellular matrix makes nonviral vectors inefficient in the areas of transfection and rapid intracellular degradation (9), which hinders the application of vectors of this kind. Several forms of nonviral vectors, including naked plasmid DNA, DNA-containing cationic liposomes, DNA-polycation complexes, DNA-phosphorylcholine polymer complexes, DNA-PLGA NPs and ODN, have been investigated as potential gene vectors for antirestenosis therapy (33,62-69) (Table 1). Chemical formulations, such as poloxamers, inhibit the endogenous DNAses, thereby protecting plasmid DNA from degradation in the extracellular matrix (70). Furthermore, dodecylated chitosan-plasmid DNA complexes formed stable,

positively charged nanospheres with small vector size and increased *EGFP-C1* gene expression in stents but not in adjacent arterial segments or distal organs, suggesting an interesting method for sustained and site-specific nonviral vector transfection (71). In addition, novel dendrimer-based poly (L-glutamic acid) derivatives have also been found to be an efficient and biocompatible gene delivery vector to inhibit restenosis in balloon-injured rabbit carotid arteries (72). Furthermore, recent studies have shown that small vector sizes in nanoscale-range particles (ie, NPs) have many advantages in DNA transfection including increased cellular uptake, decreased inflammatory response and increased cellular incorporation. This suggests NPs as a novel platform for nonviral gene vectors (73,74).

Compared with nonviral vectors, viral vectors have evolutionary advantages in their extra- and intracellular interactions. However, viral vectors can be subjected to a host of immune responses that not only negate the vector efficacy but also result in inflammatory responses *in vivo* (75). Several forms of viral vectors, including adenovirus, AAV and lentivirus, have been applied in preclinical studies and clinical trials (Table 2) (34,43,44,50,57,76-80). Adenoviruses are commonly used viral vectors because they have many features that make them well suited for gene therapy. As shown in Table 2, adenovirus-coated genes, including micro-RNA, TIMP-3 and VEGF, can have significant inhibitory effects on in-stent neointimal formation in multiple artery stent/injury restenosis animal models (34,43,44,77,80). AAV vectors have emerged as a versatile vehicle for gene delivery due to their efficient infection of dividing and nondividing cells in the presence of a helper virus (50,79). Compared with native AAVs, vascular cell-specific peptide-modified AAVs have been shown to target gene delivery to vascular tissues, such as vascular endothelial cells (ECs), *in vivo* (81). Our group has successfully transfected a novel recombinant AAV-2/9 vector with SM22 α promoter to the medial layer SMCs of swine coronary and peripheral arteries (82). It warrants mention that AAV-2/9 viral transduction in our study has no significant effect on serum amylase, fibrinogen and C-reactive protein levels, suggesting that AAV-2/9 is a safe and effective gene therapeutic vector for antirestenosis (82). Lentiviral vectors also provide a promising strategy for the treatment of cardiovascular diseases due to their durable gene transfer and their ability to govern efficiently. Several groups have verified that lentivirus-mediated gene transfection may represent a novel therapeutic approach for the prevention of restenosis after vascular interventions (57,76) (Table 2).

Vector delivery strategies

Percutaneous catheter-based delivery: Percutaneous catheter-based gene delivery, currently the most frequently used strategy for antirestenosis therapy, has several major theoretical advantages: concomitant performance with other percutaneous procedures, such as combining gene therapy with mesenchymal stem cell therapy; utilization of smaller quantities to reduce potentially harmful effects of systemic delivery (83); and easy preparation, characterization and reproducibility. However, several major limitations have also limited the application of this strategy. First, most catheters require prolonged total occlusion of the target vessel for effective vector delivery, which may increase the risk for myocardial ischemia. Second, there is lack of efficiency in site-specific gene delivery, although current vector delivery systems have focused on modified balloon catheters that can trap a fluid within a short segment of vasculature (84). Finally, the delivered viral or nonviral vectors inevitably disperse from side branches of the coronary vasculature, which carries the potential risk for distal spread.

Another promising method of transluminal gene delivery involves using vascular tissue-specific targeted systemic treatments. White et al (81) isolated human venous EC-targeting peptides and genetically incorporated them into AAV capsids. Intravenous infusion of engineered AAVs into mice caused reduced vector accumulation in the liver while enhancing uptake of virions in the vena cava (81). Deglau et al (85) investigated a site-specific delivery system. First, they injured rabbit femoral arteries using biotin molecular-loaded balloons, then intravenously

administered avidin-coated microspheres, which has a high affinity for biotin. They found that these microspheres attached to the biotin on the arterial wall, suggesting the potential to locally deliver a systemically injected antirestenotic agent (85). In addition, genetic engineering of human stem cells with VEGF holds great potential as a platform for cell-based therapy to promote vascularization and tissue regeneration (86). Nonviral, biodegradable polymeric NPs were recently developed to deliver genes to human MSCs (87), and their surface can be easily manipulated with the addition of special ligands. This may be responsible for enhancing vascular injury site-specific NP permeability, suggesting that stem cell-based delivery of gene-loaded NPs offers an interesting option to overcome such deficiency in transluminal gene delivery.

Stent-based delivery: As with DES, GES use stents as permanent scaffolds to deliver biologically active agents locally for a prolonged duration to the site of vascular disease (65). Dichek et al (88) seeded stents with genetically engineered endothelial cells using retroviral-mediated gene transfer – the first progress in the field of GES therapy to prevent ISR. In a pig coronary angioplasty model, Klugherz et al (89) reported the first successful transfection *in vivo* using a DNA controlled-release stent and a collagen-coated stent with monoclonal antibodies covalently bonded to adenovirus (90). Much later, Walter et al (65) improved the efficiency of local delivery of naked plasmid DNA encoding for human VEGF-2 via GES in hypercholesterolemic rabbits. Swanson et al (91) also observed the effect of VEGF-coated stents on restenosis in hypercholesterolemic rabbits. Currently, stent backbones designed for gene loading include biodegradable, bioabsorbable, nanoporous metal stents coated with an outer layer of polymer, which may be bioabsorbable or nonbioabsorbable material, and can be loaded with therapeutic genes, thus providing more controlled and sustained gene delivery and allowing for more optimal gene-tissue interactions (26). Non-biodegradable polymers (BPs) are currently the most frequently used method of coating the stents. However, considering that stents, similar to any other foreign material inside the human body, can cause infection and inflammation, BPs including poly-L-lactide, polyvinyl pyrrolidone, poly-lactide-cocaprolactone and PLGA, offer greater advantages. Bioresorbable stents, often composed of polylactides such as poly-L-lactide or poly-lactide-cocaprolactone, are typically completely metabolized in approximately 12 to 18 months (92). In a porcine model, Lincoff et al (26) have found that stents composed of high-molecular-weight poly-L-lactide produced minimal inflammation and durable results compared with uncoated stents. Although BP-DES have yet to receive approval in the USA, they are widely used worldwide, including in Asia and Europe. In a five-year follow-up study, Kuramitsu et al (93) investigated the long-term coronary arterial response to BP-biolimus-eluting stents (BES) compared with durable polymer sirolimus-eluting stents and BMS, and found that BP-BES shows a favourable coronary arterial response compared with sirolimus-eluting stents (93). However, in a network meta-analysis that included 113 trials involving 90,584 patients performed to compare the safety and efficacy of BPs, DES, BMS and durable-polymer DES in patients undergoing coronary revascularization (94), BP-BES was found to be associated with a higher risk for definite or probable ST compared with cobalt-chromium everolimus-eluting stents. These findings suggest the benefits of biodegradable polymer coating over a nonbiodegradable one such as durable polymer (26). However, additional studies are warranted to confirm these conclusions.

Compared with a catheter-based delivery strategy, GES represents a more appealing method for gene delivery to atherosclerotic coronary vessels. However, there are a number of technical and biological challenges with a stent-based delivery strategy. First, the limited surface area of the stent does not always guarantee the abundant loading of vectors, which is necessary for sustained transgene expression. Second, there are difficulties with regard to preservation of the mechanical properties of stents after coating the material. Third, the interaction between the biological coating material and the tissue limits the cellular uptake and intracellular stability of the gene construct, as well as

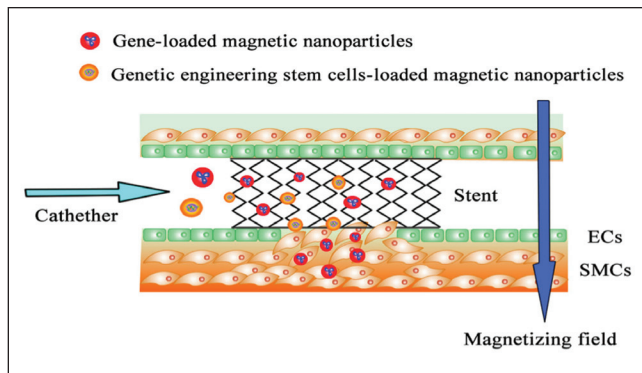


Figure 2) Schematic representation of the targeting of antirestenosis genes or genetic engineering stem cells loaded with magnetic nanoparticles driven by a magnetic field. Magnetic nanoparticle-loaded therapeutic vectors including antirestenosis genes or genetic engineering stem cells were targeted to stents and effectively transduced into endothelial cells (ECs) and smooth muscle cells (SMCs) in the artery under the presence of a uniform magnetic field

the efficient transcription and translation of the encoded protein. In addition, larger-sized particles loaded in GES may be taken up by macrophages rather than the target SMCs or ECs (6).

Magnetically guided delivery: Magnetic guidance is a physical targeting strategy with the potential to improve the safety and efficacy of a variety of therapeutic vectors (95). Magnetically guided delivery systems loaded with drug and cells to stented blood vessels has recently been proposed as a potential therapeutic strategy for in-stent restenosis (96,97). Magnetically targeted delivery systems are essentially composed of two primary components: magnetic particle-loaded therapeutic vectors and a magnetic field source generating a force attracting the vectors (Figure 2). Polyak et al (97) were the first to demonstrate high-magnetic-field gradient targeting of magnetic NP (MNP)-loaded endothelial cells to the surfaces of steel stents. At first, the investigators preloaded bovine aortic endothelial cells with biodegradable polymeric MNPs, thereby rendering the cells magnetically responsive. Then, the cells were injected via a catheter to the site advanced beyond the stent to the aortic arch. Under an external homogeneous magnetic field, the MNP-loaded bovine aortic endothelial cells were successfully targeted to the stent wires (97). Recently, Chorny et al (77) reported a novel site-specific gene delivery to stented arteries using magnetically guided delivery. In their study, zinc oleate-based MNPs were loaded with replication-deficient adenovirus, which is limited in its clinical applicability by rapid inactivation. Under magnetic conditions, adenovirus-loaded MNPs were effectively transduced into cultured ECs and SMCs. In addition, localized arterial gene expression in the magnetically guided MNP delivery group were significantly stronger than in control groups in a rat stent model, confirming the feasibility of using adenovirus-loaded MNPs to achieve site-specific transduction in stented blood vessels (77). Considering that recombinant vectors, whether viral or nonviral, must migrate through various biological barriers in one or more compartments to subsequently transfect target cells such as SMCs, an MNPs-based delivery system under a magnetic field may be seen as an exciting alternative for improving targeted antiproliferation gene release under catheter-based or stent-based delivery strategies (Figure 2).

CONCLUSION

The design of new antineointimal hyperplasia and re-endothelialization systems for the treatment of ISR was the major purpose of developing next-generation PCI technologies, which more safely and effectively prevent the progress of atherosclerosis. Vascular gene therapy offers the potential to overcome the limitations of pharmacological

interventions such as the long-term use of dual antiplatelet therapy and the side effects of antiproliferation drugs loaded in DES. Many candidate genes have the potential to prevent restenosis in various artery stent/injury animal models. However, to date, few clinical studies have demonstrated similar success rates, which may be related to the species-specific differences between human and animal models of the cardiovascular system and host-immune response. Swine coronary restenosis highly resembles restenosis in humans; thus, the swine is widely regarded as an accurate model for mimicking the proliferative component of human restenosis (98). There is a growing understanding that the physiological barriers to efficient, targeted gene transfer by gene vectors must be taken into consideration during the design of optimized therapeutic strategies. At present, viral vectors, including AAV and lentivirus, have the most potential to maximize transduction efficiency, although the toxicity, inflammation and interaction with tissue components remains to be fully evaluated. A GES-based delivery strategy is superior with regard to site-specific and sustained transgene expression. However, the stent design and material technology must be improved. The novel MNP formulation designed for magnetically guided adenoviral gene transfer to cells of the blood vessel wall addresses several prerequisites for a safe and effective gene transfection, especially targeting antiproliferation genes to SMCs. Genetic engineering of human mesenchymal stem cells using biodegradable polymeric NPs has recently been shown to enhance angiogenesis, offering an interesting option for enhancing endothelialization. These results suggest that gene-loaded NP systems and mesenchymal stem cells may be a next step for antirestenosis therapy, although the exact effect remains to be elucidated in preclinical studies and clinical trials.

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