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REVIEW ARTICLE

Delivery of viral vectors for gene therapy in intimal hyperplasia and restenosis in atherosclerotic swine

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Abstract Cardiovascular diseases including atherosclerosis are a major financial and health burden globally. Inflammation associated with atherosclerosis results in the development of plaques that can rupture causing thrombosis, stroke, or death. The most widely used treatment for the removal of atherosclerotic plaques is percutaneous transluminal coronary angioplasty (PTCA) with or without stenting. Although this is a safer and minimally invasive method, restenosis and intimal hyperplasia after interventional procedure remains a major hurdle and more refined approaches are needed. Studies in large animal models such as pigs have facilitated a greater understanding of the underlying mechanisms of the disease and provided novel targets for therapeutic intervention. In pre-clinical studies, viral vector gene therapy has emerged as a promising option for the reduction and/or prevention of restenosis and intimal hyperplasia. Although studies in animal models have generated promising results, clinical trials have yet to prove the clinical efficacy of gene therapy in coronary artery diseases. In this review, we examined and critically reviewed the most recent advances in viral vector gene therapy obtained from studies using porcine model of atherosclerosis.

Keywords Restenosis · Intimal hyperplasia · Viral vectors · Gene therapy · Porcine models

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Introduction

Cardiovascular diseases (CVD) are an immense economic and heath burden in the USA and globally. Although the death rates attributed to cardiovascular diseases declined by 25.5% between 2003 and 2013, CVDs still account for one in every three deaths in the USA [1]. Complications of atherosclerosis is the underlying cause of most clinical cardiovascular events. Atherosclerosis is a chronic inflammatory and fibroproliferative disease that results from the interaction of modified lipoproteins, endothelial cells, leukocytes, and intimal smooth muscle cells [2, 3]. Inflammation associated with the disease can result in the development of plaques that protrude into the lumen of arteries. Plaque rupture and thrombosis are the major causes of acute clinical complications of myocardial infarction and stroke as well as sudden coronary death [3, 4].

In humans, the process of lesion development associated with atherosclerosis occurs over several decades and is caused by multiple factors including genetic susceptibility, increased blood concentration of apolipoprotein B-containing lipoproteins, familial hypercholesterolemia, smoking, hypertension, and diabetes mellitus, among other factors [3, 5]. The mechanisms driving plaque formation involves lipoprotein retention, recruitment of inflammatory cells (specifically monocytes/macrophages), foam cell formation, smooth muscle cell proliferation, and immunological responses, apoptosis and necrosis, culminating in plaque stability [2–5]. Most plaques remain asymptomatic or stable while others may become unstable with increased risk of rupture thus resulting in thrombosis and acute coronary syndrome [5].

Current treatment options for unstable or vulnerable plaques are either systemic or local therapy. Systemic therapy involves the use of medications to stabilize the plaque. These include the use of low-density lipoprotein cholesterol (LDL-c) lowering therapy (statins, cholesterol absorption inhibitors);

high-density lipoprotein cholesterol (HDL-c) increasing therapy (cholesteryl ester transfer protein inhibitors); triglyceride-lowering therapy (statins, fibrates); as well as anti-inflammatory therapy for the prevention of coronary events [6, 7]. Local therapy for unstable plaques primarily employs the use of surgical procedures in cases where there is increased incidence of a possible coronary event. The most commonly used procedures for coronary artery revascularization include coronary artery bypass graft (CABG), a surgical procedure that uses vein or arterial grafts to bypass the obstruction, and percutaneous transluminal coronary angioplasty (PTCA), using a tiny balloon to inflate blocked arteries, with or without stenting [6, 8]. Despite the advances made in atherosclerosis research over the last few decades, restenosis, the renarrowing of blood vessels due to intimal hyperplasia and arterial remodeling after interventional procedures, remains a major complication to long-term success of these methods [9, 10]. In this review, we focused on recent advances in the treatment of restenosis and associated intimal hyperplasia using viral vectors as gene therapy in porcine models of atherosclerosis.

Animal models of atherosclerosis

Humans are the ideal subjects to study the pathogenesis of atherosclerosis; however, due to ethical and practical reasons, researchers have relied heavily on several types of animal models to give some insight into the underlying disease mechanisms. Animal models of arterial injury have provided invaluable information about the mechanisms regarding the arterial response to injury, interactions of the coronary artery with medical devices, as well as a better understanding of neointimal generation [11]. Several animal models including rodents, pigs, and non-human primates have been used to study restenosis and intimal hyperplasia (Table 1). In humans, disease progression typical spans over several decades. In order to facilitate research within a reasonable timeframe, animals are either placed on high-fat or high-cholesterol diets and undergo some type of mechanical injury with or without the special diet or by engineering genes involved in the clearance of lipoproteins [11, 12].

It is important to note that each animal model has its advantages and disadvantages and there is currently no perfect model for atherosclerosis (Table 1). Although rodent models have been useful in the general characterization of mechanisms underlying atherosclerosis, their small sizes and differences in cardiac anatomy when compared to humans have been the major drawback of these models [11, 13]. Given the issues with direct translational significance of rodent research to human studies, large animal models, due to their anatomical and pathophysiological similarities to human, provide a better translational bridge from pre-clinical to clinical studies. Of the large animals utilized in atherosclerosis

Table 1 Advantages and disadvantages of animal models of atherosclerosis

Species	Advantages	Disadvantages	Models/Species	References
Mice	Cheap, easy to handle, can be bred for genes of interest	Small size of arteries	LDL-KO	[11–13, 62, 63]
	Wide range of easily available and affordable antibodies and drugs	No coronary lesions	Lipase-KO	
		Wild type relatively resistant to atherosclerosis	Human ApoB100	
	Multiple inbred strains	Monotypic HDL	Human CETP expression	
	Atherosclerosis develops over a relatively short time	Differences in cells in tissues involved in arterial intima		
Rabbits	Similarities in lipoprotein metabolism in humans	Lack of hepatic lipases	WHHL (deficient in LDL receptors)	[12, 62–64]
	Express CETP	Large foam cell lesions	St. Thomas Hospital (STH)	
	Small, high availability, low-economic cost	Modest genetic variation	New Zealand White strain	
Pigs	Similar heart size and cardiac vasculature anatomy	Large size limits practicality	Rapacz (WT with mutations in ApoB and LDL gene)	[11–13, 25, 64]
	Closer genetic similarities to humans	Expensive	Diabetic hypercholesterolemia	
	Human-like lipoprotein profile (except for HDL subclasses)	Limited genetic models	PSCK gain of function	
	Similar lesion development as seen in humans	Toxic diet needed for atherosclerosis	Ossabaw	
	Large size facilitates tissue availability and non-invasive techniques			
Non-human primates	Similar cardiac anatomy	Expensive		[12, 62, 64]
	Lipoproteins and lesions like humans	No genetic modifications and limited genetic information		
	Develop coronary lesions	Specific ethical concern		

HDL high-density lipoproteins, *ApoB* apolipoprotein B, *WHHL* Watanabe heritable hyperlipidemic, *LDL* low-density lipoprotein, *CETP* cholesteryl ester transfer protein

research, the swine is currently the most relevant animal used [11–13]. Pigs are susceptible to diet-induced hypercholesterolemia like human, and the atherosclerotic lesions can be accelerated by feeding the animals high-cholesterol and/or high-fat diets. Pigs offer a large animal model with a similar cardiovascular anatomy, physiology, and closer genetic similarities to humans. Additionally, morphology and progression of lesions as well as several pathological features seen in swine models resemble those seen in human [11–13].

Pathophysiology of restenosis

PTCA was first introduced as a less-invasive alternative to CABG and is now the most prevalent method of coronary artery revascularization. Initial limitations of high rates of restenosis and arterial recoil led to the introduction of coronary stents which offered improved radial strength thus reducing rates of arterial closure and restenosis [8, 14]. Although, this is an improvement to angioplasty alone, in-stent restenosis occurs in about 30% of cases within 6–12 months of stenting [8, 14].

Injury to the coronary artery by balloon angioplasty or stent placement leads to a response of the vascular tissue. Although the mechanism of injury mediated by these processes are different, the response to the injury can be very similar [8, 9]. The early phase of restenosis is characterized by elastic recoil, which takes place due to the mechanical response of overstretching of elastic fibers of the vascular wall by balloon catheter. Deployment of stent causes partial or complete damage to the endothelial layer that results in the activation of platelets, infiltration of leukocytes, and the release of cytokines and growth factors [8–10]. Leukocyte-platelet interactions play a role in the initiation and progression of neointimal formation. Infiltrating macrophages phagocytose cell debris and secrete a host of cytokines and chemokines that facilitate the migration and proliferation of smooth muscle cells (SMCs) from the media to the intima. Medial SMCs then change phenotype from contractile to synthetic resulting in the deposition of extracellular matrix (ECM) in the intima. The ECM now allows the adhesion of macrophages and SMCs. SMCs secrete hyaluronan and proteoglycans which further enrich the ECM. This enables trapping of inflammatory cells that secrete matrix metalloproteases (MMPs) that digest hyaluronan resulting in increased types I and II collagen ultimately resulting in wound healing and fibrosis (Fig. 1) [8–10, 15].

High incidence of in-stent restenosis is one of the major drawbacks of PTCA thus creating a demand for improvement in stent technology. Drug-eluting stents (DESs) were invented

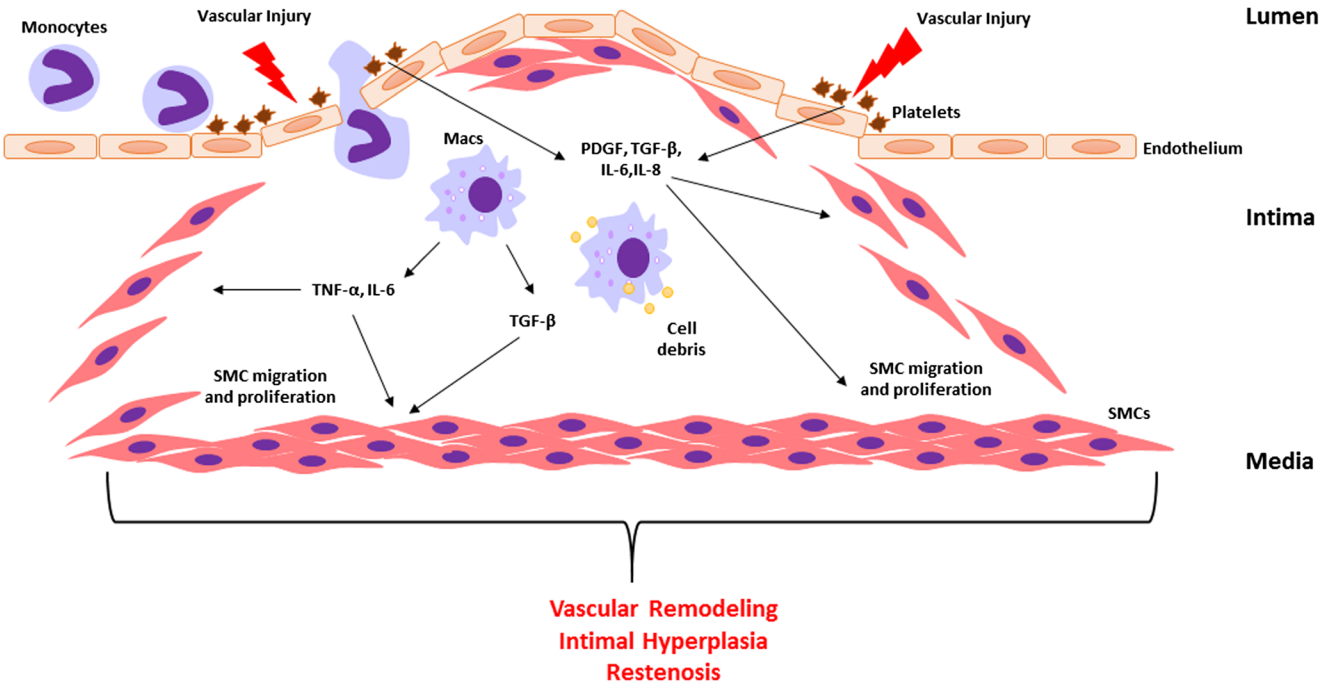

Fig. 1 Pathophysiology of restenosis: after balloon infiltration or stent deployment, there is elastic recoil. Damage caused by deployment of stent results in the activation of platelets which along with fibrin is deposited at the injured site. Activated platelets secrete several pro-inflammatory cytokines and growth factors which promote infiltration of leukocytes. Infiltrating macrophages phagocytose cell debris and secrete a variety of chemokines and cytokines. These cytokines along with those secreted from SMCs and platelets stimulate the migration of SMC from the media to the intima. SMCs then change their phenotype resulting in deposition of ECM over several weeks. Over longer time periods, the artery undergo remodeling. *PDGF* platelet-derived growth factor, *TGF* transforming growth factor, *IL* interleukin, *TNF* tumor necrosis factor, *SMCs* smooth muscle cells, *ECM* extracellular matrix, *MMP* matrix metalloproteases, *ROS* reactive oxygen species

to address this problem but certain safety concerns such as non-specificity of therapeutics, in-stent thrombosis, the need for long-term anti-platelet agents, and local hypersensitivity to polymer delivery matrices are still issues of concern [16, 17]. Improving stents, using the delivery of DNA, siRNA, and miRNA for the targeting of genes involved in plaque development offers an attractive alternative to current DESs [17]. Despite the potential benefits that may be offered by gene therapy, these alternatives warrant careful investigation to clearly elucidate mechanisms of action and potential drawbacks of each method.

Viral vectors for gene therapy

Gene therapy involves the local or systemic delivery of a specific candidate therapeutic gene to a cell or organ to treat or prevent a disease. The four main methods of introducing therapeutic genes into the vasculature include (1) ex vivo gene transfer—the delivery of therapeutic genes to target tissue in a safe and efficient manner, (2) cell-based genetic modification—ex vivo transduction from cells harvested from patients followed by re-implantation of genetically modified autologous cells, (3) local gene delivery—local delivery of genes via catheter or stents, and (4) systemic gene delivery [18]. Successful gene transfer requires the appropriate gene and a suitable vector for delivery and efficient expression of the transgene. The gene transfer vector should be non-pathogenic, should elicit minimal immunogenic response, and effectively transduce target cells. Given the multifaceted and complex mechanisms of coronary artery diseases, each gene must be tailored to a particular disease. This includes mode of delivery (intramyocardial injection, coronary perfusion, and pericardial delivery); type of vector (viral vs non-viral-based vectors); target tissue; and length of gene expression [18, 19].

Most gene transfer studies in cardiovascular diseases have utilized viral vectors namely adenoviruses (AD) and adeno-associated viruses (AAVs). AD and AAVs are advantageous over other types of viruses because they infect nondividing cells and have both been shown to be reasonably efficient at transducing the heart [18–20]. AAVs are preferred over ADs because they elicit less of an immune response enabling longer gene expression after gene transfer [19].

Gene therapy for restenosis and intimal hyperplasia

The mechanisms and inflammatory markers involved in restenosis after PTCA has been extensively reviewed [21–24]. The major players in arterial remodeling after vascular injury include MMPs, transforming growth factor (TGF)-β, nitric oxide, and oxidative stress. Restenosis gene therapy has been focused primarily on targeting markers of SMC proliferation and migration, reendothelialization, thrombosis, and oxidative damage [16, 17, 23, 24]. Current gene therapy options can be characterized as anti-proliferative (cytotoxic, cytostatic, or inhibitory genes), antithrombotic, or having mixed mechanisms based on their cellular targets and mode of action [16].

As mentioned earlier, when compared to smaller animals, pigs are a more favorable model to study the mechanisms of restenosis given their large size, the ability to spontaneously develop atherosclerosis (which can be accelerated by feeding them a high-fat and/or high-cholesterol diet), they have human-like lipoprotein profiles, and develop lesions in the coronary artery [11–13]. Current experimental models include the Rapacz familial hypercholesterolemia pig, Yucatan microswine, diabetic hypercholesterolemia pig, PCSK9 gain of function pig, and the Ossabaw (metabolic disease) pig model [12, 25]. The recent finding from viral vector gene therapy studies using pig models are reviewed in the following section and summarized in Fig. 2.

Therapeutic targets

Mediators of arterial remodeling

TGF-β1

TGF-β1, a member of the TGF-β family, is a pluripotent profibrotic cytokine that plays an important role in the pathology of vascular remodeling which is characteristic of intimal hyperplasia [22, 23]. The cytokine exerts diverse effects on several cell types involved in vascular injury namely endothelial cells, SMCs, and leukocytes [26]. Activation of TGF-β1 signaling is initiated by binding of the cytokine to TGF-β1 receptor type II (TβR-II). This then recruits and dimerizes with TGF-β1 receptor type I (TβR-I), forming a heterotrimeric complex (TGF-β1, TβR-II, and TβR-I) resulting in the activation of serine-threonine kinases of TβR-II and TβR-I which then stimulates signaling via the Smad pathway. Phosphorylated Smad2 complexes with Smad3 and Smad4 and the complex gets translocated to the nucleus where it can regulate gene expression [22, 23, 26].

The role of TGF-β1 in promoting vascular cell growth in intimal hyperplasia was first identified in the early 1990s. In a 1993 study, human TGF-β1 gene was injected in porcine arteries in vivo to investigate the role of TGF-β1 in vascular growth. It was found that expression of TGF-β1 in normal arteries resulted in ECM production which was accompanied by intimal and media hyperplasia. Immunohistochemical analyses revealed increased procollagen, collagen, and proteoglycan synthesis in the neointima compared to control-transfected arteries [27]. Subsequent studies have shown that balloon injury from angioplasty causes an early and rapid

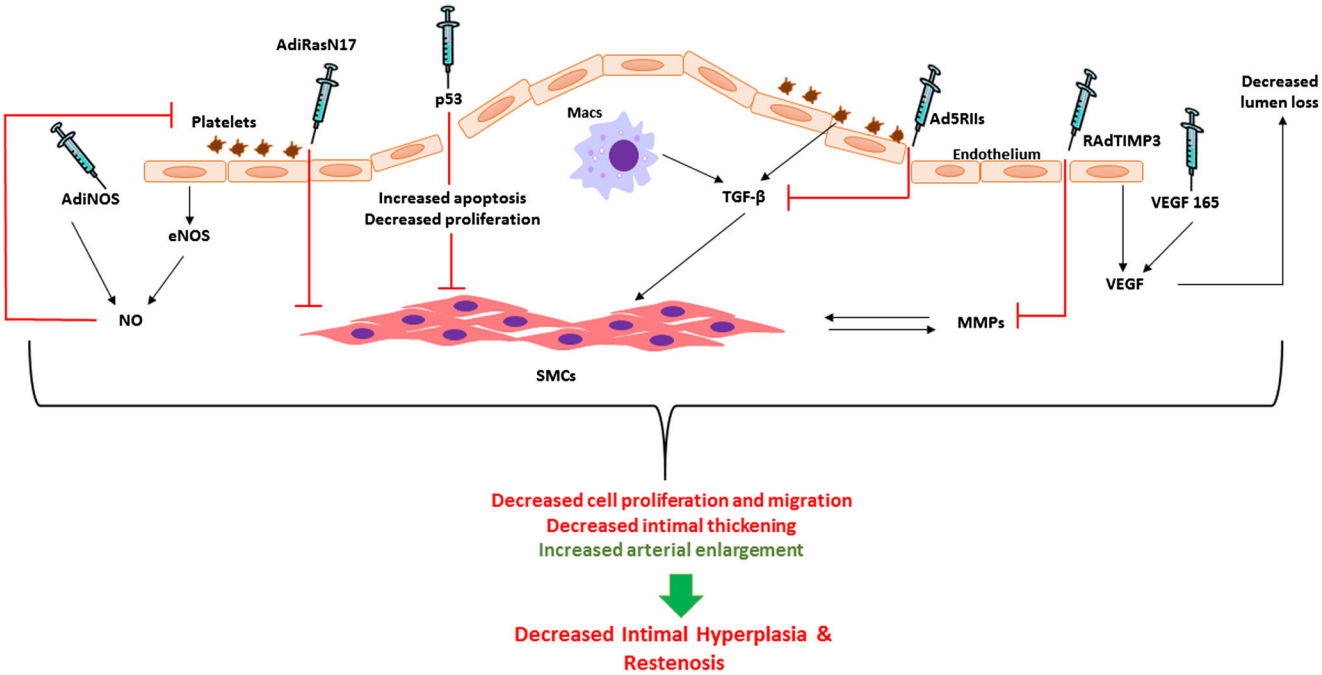

Fig. 2 Gene therapy targets for restenosis and intimal hyperplasia: gene therapy for restenosis after PTCA is characterized as anti-proliferative, primarily targeting SMC migration and proliferation, antithrombotic or having mixed mechanisms based on cellular targets. Pre-clinical studies in pig models have highlighted several gene targets using viral vectors transfer. These include iNOS, p53, TIMP-3, VEGF, Ras mutant, and TβRII. Adenoviral transfer of iNOS (AdiNOS) led to increase NO which significantly reduced neointimal hyperplasia. Administration of a Ras mutant (RasN17) led to decreased neointimal formation and increased lumen size by modulation of SMC proliferation. Overexpression of p53 has been shown to increase apoptosis and decrease proliferation. Antagonism of TGF-β using the secreted form of TβRII results in reduced luminal loss and ECM formation after angioplasty. Adenoviral delivery of TIMP-3 can reduce neointimal formation and increase apoptosis in the neointima for up to 3 months after administration. Finally, VEGF165 gene delivery resulted in reduced lumen area loss to arterial enlargement. Collectively, administration of gene via adenovirus delivery results in decreased cell proliferation and migration, decreased intimal thickening, and increased arterial enlargement ultimately decreasing intimal hyperplasia and restenosis. *iNOS* inducible nitric oxide synthase, *TIMP* tissue inhibitor of MMPs, *MMP* matrix metalloproteases, *SMCs* smooth muscle cells, *VEGF* vascular endothelial growth factor, *TGF* transforming growth factor

increase in active TGF-β levels which correlates to activators of TGF-β expression [28].

Since the establishment of its role in ECM remodeling and restenosis, TGF-β1 targeting has become the focus of several research studies. Adenovirus mediated antagonism of TGF-β, using a secreted form of TβR-II (Ad5-RIIs), at the site of PTCA was found to reduce luminal loss 28 days after angioplasty when compared to injection with adenovirus expressing β-galactosidase (Ad5-lacZ) or vehicle in porcine arteries. Antagonism of TGF-β1 also stimulated formation of a dense collagenous adventitia which acted as an external scaffold preventing constrictive remodeling [29]. In a follow-up study, the same group investigated the effects of TGF-β3, which has been shown to downregulate TGF-β1 expression and reduces TGF-β-induced ECM deposition, on luminal loss after angioplasty. Pig arteries received adenovirus expressing TGF-β3, TGF-β1, or lacZ or PBS only at the site of angioplasty and morphometric analyses were conducted 28 days' post angioplasty. Results showed that there was reduced luminal loss in TGF-β3 vessels compared with lacZ and PBS only vessels, with no reduction in TGF-β1 vessels. TGF-β3 was found to inhibit constriction remodeling after PTCA, which was accompanied by increase adventitial collagen [30]. Both studies highlight and confirm the potential of TGF-β gene therapy for the modification of ECM remodeling as a prophylaxis for restenosis.

In another experiment to determine expression pattern, inflammatory reaction, feasibility, and complications of catheter-based local adenovirus mediated gene delivery of soluble TβR-II; it was shown that this procedure was effective and feasible in a selected artery. That being said, the procedure must be undertaken with caution due to possible lethal complications [31]. More recently, the same group has shown that blockade of TGF-β by catheter-based local intravascular gene delivery reduced ECM formation to some extent but did not reduce stent-induced neointima formation at 4 weeks but induced vascular inflammation and associated pathological changes that may potentially aggravate lesion progression [32]. It is important to note that this study was not without its limitations which may account for some of the results seen.

To clarify whether gene therapy strategies to antagonize TGF-β are actually useful in coronary vasculature or just

due to differences in modes of healing of the coronary, carotid, and iliofemoral arteries after injury, porcine arteries were randomized and received custom-made CoverStent preloaded with either saline, TβR-II adenovirus, or lacZ. Upon analyses of vessels 28 days' post stenting, it was found that in-stent diameter was significantly greater and in-stent luminal loss as well as neointimal formation was reduced in TβR-II-treated arteries. There were no noticeable effects on cellular proliferation or apoptosis in TβR-II-treated arteries, which showed greater normalized neointimal/medial collagen content. Results highlight the antagonistic effects of TβR-II gene therapy on neointimal formation in injured and non-injured arteries and provide a better potential option in place of the current drug eluting stents in clinical use [33].

MMPs

MMPs are a large family of calcium-dependent zinc containing endopeptidases that function mainly to degrade ECM in various tissues. The role of MMPs in atherosclerosis have been reviewed extensively [34, 35]. Of the MMPs implicated in atherosclerosis, MMP-2 and MMP-9 are of special interest given their expression on SMCs coupled with their ability to breakdown components of the basement membrane and collagen [22, 35]. The activity of MMPs is controlled by tissue inhibitors of MMPs (TIMPs) with TIMP-1 and TIMP-2 shown to the expressed in normal arteries [34, 35]. There are studies currently investigating the effects of systemic MMP inhibition; however, a local delivery system may be more efficient at the reduction of offset targets. To that end, studies have been conducted to determine the effects of local delivery of TIMPs through the vasculature [23].

The first study to identify the regulation of MMPs by TIMPs in relation to neointimal formation was published in 1999. Using RT-PCR for messenger RNA (mRNA) expression of TIMP1, 2, and 3, it was shown that all three TIMPs were expressed before and after cell culture of human saphenous veins. TIMP-1 was shown to be the most abundant TIMP secreted with increased expression on neointimal SMCs. Rates of TIMP secretion was also found to exceed those of MMP-2 and -9 14 days in culture [36]. In another study published the following year, adenovirus delivery of TIMP-3 at the luminal surface of human and pig saphenous veins resulted in high TIMP-3 immunoreactivity in the luminal and upper medial extracellular matrix after adenovirus delivery. Apoptosis in the neointima and medial layer were significantly elevated by overexpression of TIMP-3. Neointimal formation was reduced in both human and pig vein grafts 14 days in culture [37]. Using stent eluting technology to deliver an adenovirus capable of expressing TIMP-3 (RAdTIMP-3) to pig coronary arteries, researchers showed that RAdTIMP-3 coated stents increased apoptosis and reduced neointimal cell density without increasing inflammation and proliferation as seen in RAdlacZ arteries. Neointimal area 28 days after deployment was also reduced, demonstrating the feasibility of adenovirus-coated stent technology and potential of TIMP-3 to inhibit in-stent neointima formation [38]. In a later study to determine whether recombinant adenovirus that overexpressed TIMP-3 (RAdTIMP-3) affected intimal thickening in the long-term, it was found that harvested porcine saphenous vein grafts 3 months after RAdTIMP-3 delivery had significantly reduced intimal areas compared to control [39]. These studies show that overexpression of TIMP reduces intima thickening which is sustained over 3 months highlighting the translated potential of TIMP-3 gene therapy.

Nitric oxide

Nitric oxide (NO) has received a great deal of attention as a crucial modulator of vascular disease. Studies have shown that isoforms of nitric oxide synthase (NOS), the enzyme that produces NO, have distinct roles in atherosclerosis. NO produced by endothelial NOS (eNOS), which is constitutively expressed in endothelial cells, has an atheroprotective effect inducing vascular smooth muscle relaxation, inhibiting platelet aggregation, LDL oxidation, and SMC proliferation [40]. Inducible NOS (iNOS) is minimal under physiological conditions but can be induced during infections, tumor, and chronic inflammation as seen in atherosclerosis [40, 41]. The primary focus of nitric oxide gene therapy has been to restore or improve NO levels that have been shown to be deficient in initial stages of atherosclerosis [22, 40, 41].

Using an adenoviral vector carrying the human iNOS gene, it was shown that short-term over expression of iNOS at the time of vascular injury was an effective method of increasing NO levels locally to prevent intimal hyperplasia [42]. In a similar study, intramuscular injection of adenovirus carrying the NOS complementary DNA (cDNA) (AdCMVceNOS) into pigs that had undergone angioplasty resulted in expression of functional NOS in coronary arteries. This restored NO production resulted in reduction in luminal narrowing believed to be due to the combined effects on neointimal formation and vessel remodeling after angioplasty [43]. In another study to evaluate the efficiency of local adenoviral-mediated iNOS (AdiNOS) transfer using infiltrator catheters for local delivery as well as the effects of this transfer on restenosis, it was found that iNOS gene transfer significantly reduced neointimal hyperplasia following stent injury in a porcine coronary stented model [44]. More recently, it was shown that tethering iNOS cDNA (pcDNA3.1-iNOS) to a stent was an efficient and site specific method of delivering a therapeutic gene in a pig coronary stent model. The system was shown to have long-term effects on the inhibition of restenosis and intimal proliferation 28 days after coronary stenting [45].

Anti-proliferative gene targets

VEGF

Vascular endothelial growth factor (VEGF) has crucial roles in vascular development. VEGF is a potent angiogenesis factor that is produced by a variety of cells including SMCs and macrophages. It has been shown to be expressed in atherosclerotic plaques [46] and induce pro-atherogenic changes in lipoprotein profile of murine models [47]. In a study to examine the effects of local (peri)adventitial VEGF 165 gene transfer on vascular thickening after coronary balloon injury, it was shown that the transferred gene induced adventitial neovascularization but did not increase vascular thickening or mean intima and media growth in a porcine model of arterial injury [48]. In a later study by the same group, it was found that local delivery of VEGF 165 gene after balloon injury reduced lumen area loss, which was due to arterial enlargement. Based on the mechanisms mediated by VEFG, namely enhanced adventitial elastin accumulation, reduced adventitial myofibroblast numbers and adventitial inflammation responses, the growth factor may have a potential therapeutic role in the prevention of restenosis [49].

Anti- and pro-apoptotic genes

Given the role of SMC migration and proliferation in the progression of intimal hyperplasia and restenosis, genes that promote SMC apoptosis may be viable therapeutic options for the reduction of neointimal formation. One such pro-apoptotic gene that has been studied is p53.

p53 is a tumor-suppressive protein that has been shown to have both anti-proliferative and pro-apoptotic effects. Its role in atherosclerosis has been previously reviewed by other groups. Collectively, studies suggest that p53 is activated by DNA damage and exerts its effects by regulating cell division and apoptosis within the plaque [50, 51]. Overexpression of p53 using adenoviral-mediated gene transfer in porcine saphenous vein prior to grafting resulted in significant upregulation in apoptosis and reduced neointimal proliferation at 7 days post infection. Total graft area was increased and neointimal thickening reduced at 7 and 28 days post infection. Results highlight that induction of SMC apoptosis by p53 overexpression positively influenced vein graft remodeling [52].

More recently, another study was designed to determine if long-term enhanced external counterpulsation (EECP), a non-surgical, mechanical method of increasing blood flow to the arteries, could protect vascular endothelial cells from apoptosis by modifying expression of apoptotic-related genes. It was found that EECP could offer some protection to endothelial cells by delaying the progression of lesions via downregulation of pro-apoptotic gene Apaf-1 and upregulation of anti-apoptotic gene BIRC2 [53]. Studies highlight two genes, which could be useful targets for gene therapy in atherosclerosis pending further investigation.

Ras mutant

Ras proteins are key transducers of mitogenic signals that have been extensively studied in the development and progression of cancers. The family members are essential components of cell signaling control, cell proliferation, differentiation, and survival. Early in vivo rodent studies have demonstrated that Ras plays a key role in SMC proliferation [54] and a recombinant adenovirus expressing a Ras mutant (AdiRasN17) was able to inhibit angioplasty-induced stenosis by modulation of SMC proliferation [55]. For more translational results, these studies were repeated in pig models and it was found that administration of mutant RasN17 led to decreased neointimal formation and increased lumen size, highlighting a role of the Ras mutant as a potential gene therapy option in the prevention of neointimal formation in coronary arteries [56].

Collectively, results from pig models have identified several targets for gene therapy and have employed the use of adenoviral vectors to modulate these targets. Overall, the results have been promising and adenoviral mediated delivery of target genes have been found to be safe, efficient, and effective at ameliorating hallmark features of intimal hyperplasia and restenosis after vascular injury. However, there is still more work to be done considering that some clinical trials have yet to support the results seen in animal models.

Viral vectors for gene therapy—clinical trials

One of the earlier clinical trials to utilize adenovirus gene therapy for the prevention of in-stent restenosis was the Kuopio Angiogenesis Trial (KAT). Patients were given stents after PTCA with either VEGF adenovirus, VEGF plasmid liposome, or Ringer's lactate for control groups. In the 6-month follow-up, it was found that gene transfer with adenovirus or plasmid liposome could be performed without any major gene transfer-related adverse effects. There were no differences in clinical restenosis rate or minimal lumen diameter; however, increased myocardial perfusion was seen in VEGF-Ad treated patients [57]. In an 8-year follow-up study to determine the long-term effects of local AdVEGF gene transfer, it was found that although eight patients died during follow-up studies, there were no significant differences in mortality between the VEGF adenovirus vs VEGF plasmid vs placebo groups. Incidences of major adverse effects such as cardiovascular events, cancers, or diabetes did not differ among the groups. Results further highlight the safety and efficacy of local intracoronary adenoviral mediated VEGF gene transfer as a viable treatment option for restenosis [58].

Viral vectors have also been used to treat other coronary events, such as peripheral artery disease, where conflicting results have been found. In one trial, a single intramuscular administration of AdVEGF121 was not associated with improvements in exercise performance or quality of life [59]. Conversely, other trials have found more favorable results [60]. Other gene therapy trials for in-stent restenosis have employed the use of non-viral transduction methods. However, given that histological analyses cannot be done on live patients, it is difficult to ascertain the effectiveness of such methods [61].

Results from pre-clinical and some clinical trials have highlighted a therapeutic role of viral vector-mediated gene therapy for restenosis and associated intimal hyperplasia. There is, however, much more research needed aimed at better understanding the molecular pathways involved in restenosis as well as improvement to current viral vectors to better target the pathways driving pathological remodeling after PTCA and stenting.

Conclusions and future directions

Coronary artery intervention using ballon angioplasty and intravascular stenting is currently the safest and most widely used method to revascularize occluded arteries. However, the development of intimal hyperplasia and in-stent restenosis continues to be major adverse effects. The use of large animals like pigs has facilitated enhanced translational value of preclinical studies; however, some of these models represent a monofactorial etiology which may limit generalization of results since atherosclerosis and post-interventional arterial remodeling are multifaceted conditions. The development of more human-like porcine models encompassing multiple factors that promote atherosclerosis will contribute immensely to our growing understanding of the underlying mechanism of the disease and foster research aimed at improving current therapeutic options.

To date, several attempts have been made to improve outcomes after PTCA. The use of gene therapy to target-specific mediators of in-stent restenosis has gained attention over the last decade. The goal of gene therapy is to modify a gene or genetic pathway to provide therapeutic value and prevent and/or reduce a disease. Safety, efficiency, and efficacy of gene transfer is crucial for the treatment of human diseases. These factors have all been major drawbacks to the treatment of CVDs using gene therapy. Currently, viral vectors, more specifically ADs and AAVs, have been the vectors of choice due to low toxicity and associated inflammation as well as decreased interaction with circulating blood components. Given the multifaceted nature of restenosis and intimal hyperplasia, the ideal gene therapy strategy would be one that is capable of inhibiting SMCs proliferation while promoting regeneration of the injured endothelium. This may not be a single gene but more likely a cocktail of therapeutic genes that can achieve these desired effects.

Although there have been several advancements in the understanding and treatment of atherosclerosis, there is still much more to be done. Finding ways to improve on current therapeutic targets and delivery methods as well as discovering and developing novel ones will no doubt result in enhanced safety, efficacy, and efficiency of gene therapy.

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Compliance with ethical standards

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