Adventitial growth factor signalling and vascular remodelling: Potential of perivascular gene transfer from the outside-in

Richard C.M. Siow⁎, Adrian T. Churchman

Abstract

The adventitial segment of the vessel wall has received limited attention compared to the endothelium, media and neointima in processes involved in vascular remodelling during atherogenesis, coronary artery bypass graft failure and in response to angioplasty. The adventitia has been regarded as a relatively ‘inert’ layer providing a supportive connective tissue and extracellular matrix scaffold around vessels for nerves and the vasa vasorum. We and others have recently demonstrated that functional changes in cells within the adventitia contribute to vascular remodelling through the activation and migration of adventitial myofibroblasts, partly under the influence of transforming growth factor-β1 and platelet derived growth factor-BB. These cytokines stimulate local accumulation of progenitor cells, angiogenesis, matrix deposition and enhanced generation of reactive oxygen species, together contributing to intimal hyperplasia in vascular diseases. This review summarises the evidence that growth factors acting locally in the adventitia can influence vascular function. Furthermore we highlight the therapeutic potential of perivascular gene transfer approaches from the ‘outside-in’ to antagonise growth factor activity and to modulate expression of vaso- and redox-active genes which act in concert to prevent the progression of vascular diseases in which adventitial cells are activated.

Keywords: Adventitia; Atherosclerosis; Restenosis; Coronary artery bypass graft; Growth factor; Vascular remodelling; Progenitor cells; Smooth muscle cells, Myofibroblasts; Reactive oxygen species; Antioxidant genes; Gene therapy

1. Introduction

The capacity of the adventitia to influence neointima formation and vascular remodelling has received increasing attention over the past decade [1,2]. Histological delineation of the compartments of the arterial wall is largely historical, with the adventitia being regarded as a relatively inert layer providing a scaffold comprising of extracellular matrix and connective tissue for sympathetic nerve endings and the vasa vasorum, while the medial and intimal layers were considered to be more active participants in atherosclerosis and restenosis [3]. However, several studies have recently provided convincing evidence that functional changes in the adventitial compartment also contribute to vascular remodelling during atherogenesis, coronary artery bypass vein graft failure and following angioplasty through the activation, differentiation and luminal migration of adventitial fibroblasts, thereby contributing to neointima formation [4–6]. Several processes and pro-inflammatory factors have been proposed to act locally to contribute to the activation of the adventitial compartment, ranging from enhanced growth factor activity [7] and increased extracellular matrix synthesis [8] to generation of reactive oxygen species [9] and accumulation of progenitor cells [10].

During the response to vascular injury and inflammation, adventitial cells can be reprogrammed to differentiate into...
different phenotypes. In models of balloon injury, perivascular fibroblasts can be converted into ‘smooth muscle’-like cells, having a migratory myofibroblast phenotype [4–6,11,12]. Both adventitial myofibroblasts, and smooth muscle cells (SMC) in the medial and intimal layers are able to undergo functional and structural changes following vascular injury [13,14], reminiscent of both tissue repair processes and vascular morphogenesis during development. Given the recent studies demonstrating that progenitor cells in the adventitia contribute to the progression of atherosclerosis and restenosis [15], it is conceivable that the mechanisms leading to their homing and differentiation are also responsible for the activation and migration of resident adventitial fibroblasts. Therefore, the approach to deliver genes to the perivascular surface of blood vessels to modulate local pro-inflammatory processes responsible for adventitial activation provides a promising therapeutic strategy for vascular diseases. Moreover, perivascular gene transfer maintains the structural integrity of the vessel wall and endothelium, while high concentrations of gene delivery vectors can be maintained over longer periods in the local environment compared to luminal or systemic gene delivery techniques [16]. This brief review of studies that demonstrate the adventitial cell activation in experimental models of vascular disease highlights the potential benefits of perivascular gene transfer to reduce remodelling of the vessel wall in atherosclerosis and restenosis. In particular, we focus on the antagonism of growth factor signalling and modulation of redox state in the adventitia. In particular, we focus on the antagonism of growth factor signalling and modulation of redox state in the adventitia. In particular, we focus on the antagonism of growth factor signalling and modulation of redox state in the adventitia. In particular, we focus on the antagonism of growth factor signalling and modulation of redox state in the adventitia. In particular, we focus on the antagonism of growth factor signalling and modulation of redox state in the adventitia.

2. Growth factors in adventitial myofibroblast migration

Percutaneous transluminal coronary angioplasty in humans for the treatment coronary artery occlusion often results in restenosis, affecting more than 30% of patients who undergo balloon angioplasty without stenting. The processes leading to restenosis are similar to those that contribute to accelerated atherosclerosis and vein graft failure after coronary artery bypass surgery [17,18]. Experimental vascular injury in animal models and angioplasty in humans result in migratory and proliferative responses of SMC within the vessel wall in addition to matrix remodelling, characteristic events observed in restenosis and atherosclerosis [17]. Over the past decade, the roles of transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor BB-chain (PDGF-BB) in SMC migration and proliferation have been well characterised in atherogenesis and vascular remodelling following angioplasty in humans and models of arterial injury in animals [19,20]. Antagonism of vascular PDGF-BB signaling, through its sequestration by specific antibodies or inhibition of PDGF β-receptor kinase activity, has been demonstrated to limit vascular remodelling after balloon angioplasty in rats [21–23]. Similarly, removal of TGF-β1 or blockade of its actions in the vessel wall have also been shown to reduce neointimal hyperplasia and loss of lumen diameter following experimental vascular injury in rodent models [24–26]. These studies employed either systemic delivery of neutralising antibodies against the growth factors [21,25] or adenoviral gene delivery systemically or through the arterial lumen using vectors coordinating expression of soluble receptors to either PDGF-BB [23] or TGF-β1 [24,26]. More recently, connective tissue growth factor (CTGF) has also been implicated in enhanced adventitial myofibroblast activation and migration in a rabbit vein graft model of vascular remodelling [27] and may thus act as an additional profibrotic perivascular growth factor in synergism with TGF-β1 in the adventitia [28]. In a similar manner, vascular endothelial growth factor (VEGF) can enhance the activity of PDGF-BB to induce SMC migration and proliferation in vascular remodelling [29] and therefore represents another potential perivascular gene target.

By employing a perivascular adenoviral gene delivery technique to specifically label endogenous adventitial cells with the marker gene β-galactosidase prior to vascular injury, we have provided additional evidence to demonstrate that the phenotypic modulation and migration of adventitial myofibroblasts do contribute to neointima formation and vascular remodelling in the rat carotid artery at 7 to 14 days following balloon angioplasty [12]. Previous studies showing migration of adventitial cells in porcine coronary [6] and rat [30] carotid arteries have employed indirect techniques based on labelling cells in a replicative cycle with bromodeoxyuridine which does not specifically identify cells solely of adventitial origin. Although Li et al. [5] attempted to overcome this by seeding exogenous fibroblasts on to the perivascular surface of rat carotid arteries following endoluminal injury, cells observed migrating towards the lumen would have been culture expanded and transfected in vitro using retroviruses to express β-galactosidase, a process likely to have activated and induced phenotypic modulation of the myofibroblasts [31], prior to their reintroduction in different animals, thereby making them more likely to migrate. Therefore, our approach of only labelling resident adventitial fibroblasts in vivo by perivascular gene transfer [12] represents an efficient method of tracking the migration and phenotypic modulation of adventitial cells for over 14 days and moreover, potentially facilitates delivery of transgenes to the medial and neointimal compartments of the vessel wall through luminal migration of transfected adventitial cells following angioplasty.

A similar observation can be made of the recent study by Hu et al. [32] in which progenitor cells expressing stem cell antigen-1 (Sca-1) were isolated from the adventitia of aortic arches and roots from mice, culture expanded and differentiated in vitro by treatment with PDGF-BB for 3 days prior to transfer on to the adventitial surface of vena cava, which were then grafted on to donor mouse carotid arteries. As it has been reported that progenitor cells cultured in growth factor-enriched media can exhibit specific SMC markers such as α-smooth muscle actin, myosin heavy chain and
that local antagonism of either TGF-β transgene expression was maintained for at least 14 days and prior to vascular injury. We demonstrated that adventitial with either the AdSmad7 or AdPDGFXR vectors 4 days expression of the marker transgene the perivascular surface of an adenovirus co-ordinating phenotypic modulation of fibroblasts involved co-delivery to [42]. Our approach to determine whether growth factor [39], an early event in the classical TGF-β homologues TGF-β, type II receptor mediated Smad2 phosphorylation [39], an early event in the classical TGF-β1 signalling cascade [40,41]. Antagonism of PDGF-BB signalling in the adventitia was achieved by perivascular gene transfer using an adenoviral vector coordinating expression of the soluble extracellular region of the PDGF β-receptor (AdPDGFXR), which has been shown to bind PDGF-BB with high affinity, but not PDGF-AA, and thereby acts as a selective antagonist [42]. Our approach to determine whether growth factor antagonism in the adventitia altered the migration and phenotypic modulation of fibroblasts involved co-delivery to the perivascular surface of an adenovirus co-ordinating expression of the marker transgene β-galactosidase together with either the AdSmad7 or AdPDGFXR vectors 4 days prior to vascular injury. We demonstrated that adventitial transgene expression was maintained for at least 14 days and that local antagonism of either TGF-β1 [37] or PDGF-BB [38] attenuated adventitial cell migration to the media and neointima (Fig. 1), diminished neointimal hyperplasia and constrictive remodelling after angioplasty. Overexpression of both transgenes was associated with a decrease in the transition of adventitial cells to a SMC phenotype and deposition perivascular collagen following vascular balloon injury. However, it is possible that by restricting Smad7 or PDGFXR gene transfer to the adventitia in this study, the contribution of medial SMC proliferation to neointimal hyperplasia remained unaffected. Nevertheless, our studies and others summarised in Table 1 provide direct evidence that adventitial cells play a significant role in vascular remodelling processes under the influence of these growth factors and that perivascular gene transfer is effective in

3. Perivascular gene transfer to antagonise growth factor signalling

Our studies have employed perivascular adenoviral gene transfer strategies to locally antagonise either TGF-β1 [37] or PDGF-BB [38] signalling in the adventitial compartment prior to vascular injury involving balloon catheter mediated endothelial denudation and mechanical stretch of rat common carotid arteries. Both growth factors have been established as key mediators involved in fibroblast and SMC phenotypic modulation, migration, and hyperplasia in the pathogenesis of atherosclerosis and restenosis [17,18], however a paucity of information exists on their local actions on fibroblasts or progenitor cells in the adventitia. TGF-β1 mediated signalling in the adventitia was attenuated through inhibition of the ‘mothers against decapentaplegic homologues’ (Smad) signalling pathway by adenoviral gene transfer of Smad7 (AdSmad7), the endogenous inhibitor of TGF-β type II receptor mediated Smad2 phosphorylation [39], an early event in the classical TGF-β1 signalling cascade [40,41]. Antagonism of PDGF-BB signalling in the adventitia was achieved by perivascular gene transfer using an adenoviral vector coordinating expression of the soluble extracellular region of the PDGF β-receptor (AdPDGFXR), which has been shown to bind PDGF-BB with high affinity, but not PDGF-AA, and thereby acts as a selective antagonist [42]. Our approach to determine whether growth factor antagonism in the adventitia altered the migration and phenotypic modulation of fibroblasts involved co-delivery to the perivascular surface of an adenovirus co-ordinating expression of the marker transgene β-galactosidase together with either the AdSmad7 or AdPDGFXR vectors 4 days prior to vascular injury. We demonstrated that adventitial transgene expression was maintained for at least 14 days and that local antagonism of either TGF-β1 [37] or PDGF-BB [38] attenuated adventitial cell migration to the media and neointima (Fig. 1), diminished neointimal hyperplasia and constrictive remodelling after angioplasty. Overexpression

![Fig. 1. Perivascular gene transfer of Smad7 or PDGFXR reduces adventitial myofibroblast migration to the neointima following balloon angioplasty. To assess adventitial cell migration, adenoviral pairs encoding expression of the inactive control marker genes β-galactosidase (Adβ-gal) and green fluorescent protein (AdGFP), Adβ-gal and Smad7 (AdSmad7) or Adβ-gal and soluble extracellular region of the human PDGF β-receptor (AdPDGFXR), were suspended in pluronic gel (each at 10^10 pfu/ml) and applied to the perivascular adventitial surface of rat common carotid arteries. Four days following gene transfer, balloon catheter injury was performed in transfected carotid arteries to elicit endothelial denudation and arterial distension. Localisation of blue β-galactosidase (β-gal) positive nuclei of adventitial cells contributing to the medial and neointimal compartments were visualised following X-gal staining in sections excised at 3, 7 or 14 days after balloon injury and the proportion of β-gal positive nuclei in the neointimal compartment were quantified using chromagen separation PC software in photomicrographs of AdGFP and Adβ-gal (hatched bars) or AdSmad7 and Adβ-gal (A, solid bars) or AdPDGFXR and Adβ-gal (B, solid bars) transfected carotid artery sections. Data adapted from references 35 and 36 and represent mean±SEM, n=6–12 animals per treatment time point, ★p<0.05.](Image)
Rat Carotid artery perivascular gene transfer of adenovirus encoding...[565]

**Table 1**
Effects of perivascular gene/progenitor cell transfer on vascular remodelling

<table>
<thead>
<tr>
<th>Species</th>
<th>Study design</th>
<th>Summary of findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Carotid artery perivascular gene transfer with adenovirus encoding the NAD(P)H oxidase inhibitory peptide gp91ds. Angiotensin II (750 μg/kg/day) infused by osmotic mini pump</td>
<td>gp91ds localized to adventitial fibroblasts and angiotensin II–induced medial hypertrophy ↓ ~ 45%. Levels of lipid peroxidation (HNE formation) ↓ ~ U95% after gp91ds gene transfer indicating decreased reactive oxygen species production</td>
<td>[74]</td>
</tr>
<tr>
<td>Rat</td>
<td>Vascular injury induced by balloon angioplasty. Perivascular transfer of adenovirus coordinating expression of PDGFβR-gp91ds</td>
<td>Angioplasty ↑ intima/media ratio (I/M). Adenoviral gp91ds expression ↓ I/M area ratio ~ 80%. Reactive oxygen species ↑ ~ 6-fold in control distented arterial rings. Expression of gp91ds ↓ ROS production by 66% compared to control and ↓ hyperplasia and fibroblast proliferation by 26%</td>
<td>[73]</td>
</tr>
<tr>
<td>Rat</td>
<td>Carotid artery perivascular gene transfer of adenovirus encoding Smad7 at 4 days prior to vascular injury by balloon angioplasty</td>
<td>Smad7 overexpression ↑ adventitial cell migration to the neointima and luminal loss after balloon injury at 7 and 14 days after injury</td>
<td>[37]</td>
</tr>
<tr>
<td>Rat</td>
<td>Carotid artery perivascular gene transfer of adenovirus encoding AdPDGFRXR at 4 days prior to vascular injury by balloon angioplasty</td>
<td>PDGFRX expression ↑ adventitial cell migration to the neointima at 7 and 14 days after injury and ↑ lumen area 21%. Intimal thickness ↓ ~ 50% at 7 days and ↓ 33% at 14 days with 40% ↓ in I/M ratio at 14 days. MMP-2 and collagen expression ↓ in AdPDGFRX transfected arteries</td>
<td>[38]</td>
</tr>
<tr>
<td>Rat</td>
<td>Carotid artery injury by balloon angioplasty. Recombinant TGF-βRII injected to the tail vein every other day up to 14 days after injury</td>
<td>TGF-βRII localized to the adventitia and neointima of injured carotid artery and ↓ lesion formation (<del>65%), lumen area ↑ (</del> 88%) and I/M ratio ↓ (~ 70%). Collagen and α-actin expression ↓ in adventitia</td>
<td>[26]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Diet containing 1.5% cholesterol for 1 week prior to collar placement around carotid artery. Perivascular liposomal VEGF transfection</td>
<td>Perivascular VEGF gene transfer ↑ adventitial collar-induced neointimal thickening ~ 50% and macrophage accumulation ↓ ~ 80%. Neovascularisation not significantly increased. Endothelial cell expression of VCAM-1 ~ ↓ 70%</td>
<td>[52]</td>
</tr>
<tr>
<td>Pig</td>
<td>Balloon angioplasty in two major coronary arteries. Perivascular VEGF plasmid injection complexed with liposomes</td>
<td>VEGF gene transduction caused significant ↓ luminal loss by 2 fold at 14 and 28 days after injury. Adventitial microvessel area density ↑ at 14 and 28 days (3.5–4.3 fold). Elastin but not collagen significantly ↑ at 14 and 28 days. Expression of α-actin ↓ at 14 and 28 days by 44% and 32% in the adventitia</td>
<td>[53]</td>
</tr>
<tr>
<td>Mouse</td>
<td>ApoE deficient mice vena cava grafted on to carotid arteries. Sca-1 positive adventitial progenitor cells differentiated in vitro with PDGF-BB and transferred to adventitia of vein grafts and hyperplasia monitored</td>
<td>Transferred Sca-1 positive donor adventitial cells found within neointima and enhanced hyperplasia of vein graft lesions at 2–4 weeks after grafting indicating progenitor cells exist in the adventitia that can differentiate into smooth muscle cells that contribute to atherosclerosis</td>
<td>[32]</td>
</tr>
</tbody>
</table>

Abbreviations: gp91ds, peptide inhibitor of NAD(P)H oxidase; HNE, 4-hydroxynonenal, PDGFβR-gp91ds, peptide inhibitor of NAD(P)H oxidase driven by the fibroblast PDGFβ-receptor promoter; ROS, reactive oxygen species; AdPDGFRX, adenoviral vector encoding soluble extracellular region of the platelet derived growth factor β-receptor; TGF-βRII, transforming growth factor-β type II receptor; VEGF, vascular endothelial growth factor; VCAM-1, vascular cell adhesion molecule-1; Sca-1, stem cell antigen-1.

locally antagonising the effects of TGF-β1 or PDGF-BB on fibroblast activation. (Fig. 2).

### 4. Role of adventitial progenitor cells in vascular remodelling

It is entirely conceivable that perivascular gene transfer strategies would also diminish the homing and maturation of progenitor cells in the adventitia to a myofibroblastic phenotype since both TGF-β1 and PDGF-BB have been reported to be involved in the differentiation of human multipotent adult progenitor cells into functional SMC or myofibroblasts capable of enhancing the adventitial contribution to neointimal hyperplasia in vascular diseases [15,32,43–45]. It is also possible that adventitial progenitor cells may additionally differentiate either into pericytes [46] or endothelial cells [47] under the influence of TGF-β1, PDGF-BB or VEGF, which in turn, are likely to enhance perivascular angiogenesis [48]. It is possible that progenitor cells are either resident within the adventitia and/or recruited to the vessel wall under the influence of inflammatory cytokines and growth factors and the mechanisms involved in progenitor cell homing and differentiation have already been extensively reviewed elsewhere [15,44,47,49]. In the context of atherosclerotic plaque progression, neovascularization of the lesion, predominantly thought to arise from the adventitia where there are abundant existing vasa vasorum, contributes to intraplaque hemorrhage in advanced lesions which promotes plaque destabilisation and rupture [50]. The importance of angiogenesis is dependent on the stage of lesion development, being a trigger in the early phase and reeding within advanced lesions. Khurana et al. [51] demonstrated that adventitial angiogenesis can augment neoointima formation in both the rabbit collar and rat angioplasty models of carotid artery injury. In this study, perivascular gene transfer of an adenoviral vector encoding a...
dominant-negative fibroblast growth factor (FGF) receptor-1 significantly attenuated adventitial angiogenesis in response to vascular injury. Interestingly, liposome mediated adventitial VEGF gene transfer inhibited carotid artery perivascular collar-induced neointimal hyperplasia, macrophage accumulation, and VCAM-1 expression without a significant increase in adventitial neovascularization in a high cholesterol rabbit model of atherosclerosis [52]. In a porcine model of coronary artery angioplasty, liposomal VEGF gene transfer to the periadventitial space resulted in reduced lumen loss due to positive remodelling, prevention of adventitial microvessel regression, enhanced adventitial elastin accumulation and reduced adventitial myofibroblast numbers at 14–28 days after injury [53]. However, the study also reported a pronounced adventitial inflammatory response which may constitute arterial ‘healing’ and thus may be detrimental to perivascular remodelling, activation of myofibroblasts and cytokine generation in the long term.

In addition, the supply of oxygen and entry of inflammatory mediators such as macrophages and oxidized lipoproteins through microvessels within the adventitial compartment are important in both the progression and regression of atherosclerotic and restenotic lesions following intimal and medial hyperplasia [54]. It has been demonstrated that circulating progenitor cells not only promote endothelialization of porcine vein grafts, but also stimulate neointimal hyperplasia, suggesting that they can act simultaneously to suppress remodelling through endothelial repair but also enhance neointimal formation through differentiation into myofibroblasts in vascular diseases [55]. Identification of the homing mechanisms responsible for recruitment of progenitor cells to the adventitia of damaged vessels leading to their differentiation into myofibroblasts or promotion of angiogenesis within the vessel wall would be necessary to develop therapeutic approaches for targeting these processes. Therefore, formation of vasa vasorum-derived adventitial microvessels are an integral component of vascular disease progression [56]. Whilst the exact mechanisms by which TGF-β1, PDGF-BB, FGF and VEGF act alone or in synergy to promote perivascular neovascularisation in addition to cell migration remain to be fully elucidated, it is conceivable that perivascular gene transfer strategies can be employed to either specifically promote or inhibit angiogenic processes locally in the adventitia.

5. Adventitial extracellular matrix and vascular remodelling

An imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) contributes to vessel remodelling in restenosis [57,58]. This may be mediated in part by facilitation of adventitial cell migration through degradation extracellular matrix proteins by enhanced MMP activity and decreased TIMP expression [57]. It has been shown that perivascular adenoviral gene delivery of TIMP-2 to the adventitial surface of mouse vena cava grafted on to mouse common carotid arteries inhibits MMP activity and significantly reduces graft neointimal hyperplasia at 2 to 4 weeks after grafting [59], while non-viral perivascular

---

Fig. 2. Schematic diagram depicting activation of and migration of adventitial cells during restenosis or vein graft failure. Adventitial cell migration is initially under the influence of perivascular TGF-β1 or PDGF-BB which elicits phenotypic modulation of adventitial fibroblasts or progenitor cells to myofibroblasts and mediates their migration towards the lumen with concomitant increases in matrix metalloproteinase (MMP) expression, collagen deposition and generation of reactive oxygen species (ROS) leading to neointimal hyperplasia and loss of luminal area. Perivascular adenoviral gene transfer to overexpress Smad7 (AdSmad7) or the soluble truncated PDGF β-receptor (AdPDGFXR) antagonises TGF-β1 or PDGF-BB signalling in the adventitial compartment respectively resulting in attenuation of adventitial fibroblast or progenitor cell migration, decreases MMP expression collagen deposition and ROS generation, thereby attenuating intimal thickening and loss of luminal area following vascular injury or vein graft failure. Perivascular gene transfer of endogenous antioxidant defence genes reduces ROS/oxidative stress-mediated adventitial cell activation and migration.
delivery of TIMP-1 plasmid DNA using lipofectin and integrin-targeting peptides reduced constrictive remodelling of rat carotid arteries at 14 and 28 days following angioplasty [60]. Antagonism of PDGF-BB or TGF-β1 activities in our studies by perivascular AdPDGFXR or AdSmad7 gene transfer respectively, diminished MMP-2 and enhanced TIMP-1 and TIMP-2 expression in the adventitial and medial compartments of balloon injured arteries and decreased collagen deposition [37,38]. Through their regulation of extracellular matrix degradation, MMPs and TIMPs are additional mediators of adventitial myofibroblast and progenitor cell migration through the vessel wall and the contribution of advential cells to vascular remodelling in atherosclerosis and restenosis [57,58]. Modulation of MMP/TIMP expression through perivascular PDGFXR or Smad7 gene transfer may partially account for the attenuation of adventitial cell invasion towards the lumen observed in our studies [37,38] following rat carotid artery balloon injury since both PDGF-BB and TGF-β1 have also been shown to alter MMP and TIMP expression in cultured vascular SMC [61] and fibroblasts [62] respectively. Modulation of adventitial MMP/TIMP activity directly or through antagonism of growth factor signalling by perivascular gene transfer represents a potential strategy to limit remodelling in vascular diseases.

6. Adventitial oxidative stress and vascular remodelling

Far from being an ‘inert’ compartment of the vessel wall, adventitial fibroblasts have been shown to be an source of reactive oxygen species (ROS) in blood vessels following mechanical stretch and injury [9,63]. The literature covering the generation and actions of ROS in the pathogenesis of vascular diseases and angiogenesis has been extensively reviewed elsewhere [64–67]. Although it has been established that balloon angioplasty in rabbit [68], porcine [63] and rat [9,69] models of vascular injury results in redox imbalance, there remains a paucity of reports relating to the contribution of adventitial sources of ROS and the benefits of antioxidant gene expression in vascular remodelling. Superoxide, generated by NAD(P)H oxidase in response to angiotensin II (10 pM) in the adventitia of wild-type but not NAD(P)H oxidase subunit gp91phox deficient mouse aortas, has been shown to contribute to impaired endothelium-derived nitric oxide (NO)-dependent relaxation which was reversed by local adventitial application of superoxide dismutase (SOD) [70]. In this context, perivascular gene delivery of an adenovirus encoding endothelial nitric oxide synthase (eNOS) in canine arteries resulted in adventitial fibroblast NO generation [71] which would beneficially restore vascular reactivity in arteries exhibiting diminished endothelial function [72]. Specific inhibition of adventitial NAD(P)H oxidase activity by perivascular gene transfer using an adenoviral vector encoding gp91ds, a peptide inhibitor driven by the PDGF-β1 receptor promoter targeted to fibroblasts and interfering with the interaction between NAD(P)H oxidase subunits gp91phox and p47phox, has been demonstrated to reduce vascular superoxide generation and neointima formation in rat and mouse carotid arteries 14 days after balloon angioplasty [73,74].

It is noteworthy that PDGF-BB has been shown not only to enhance superoxide generation in cultured vascular SMC via NAD(P)H oxidase activity [75], but it also modulates expression of peroxiredoxin-II, an endogenous hydrogen peroxide scavenging antioxidant gene [76], in a murine model of vascular injury [77], thus providing additional evidence to support an additional ‘pro-oxidant’ potential of adventitial PDGF-BB signalling in restenosis. Furthermore, TGF-β1 has been recently reported to induce ROS generation and expression of Nox4, an NAD(P)H oxidase homolog, in cultured human arterial SMC through a Smad2 dependent signalling pathway [78]. In addition, the possibility exists that activation of latent TGF-β1 can be directly mediated by ROS, through a redox switch recently identified as a methionine residue at amino acid position 253 [79]. This suggests that latent TGF-β1 sequestered in the adventitial extracellular matrix may be activated not only by increased MMP-2 activity [80] but in addition by enhanced adventitial myofibroblast ROS generation, contributing to augmented perivascular TGF-β1 activity during vascular remodelling [7,37,81].

7. Antioxidant genes and inhibition of vascular remodelling

Given that growth factors can additionally elicit ROS generation by vascular cells [75,78], experimental models of restenosis and bypass graft failure suggest that antioxidant therapies can also be effective to ameliorate vascular remodelling [82,83]. The dietary antioxidant vitamin E has been reported to significantly reduce neointimal thickening in response to balloon injury in rat carotid arteries [84], while more recently, the antioxidants N-acetyl-cysteine and tempol have been demonstrated to reduce PDGF β-receptor activation by enhancing protein tyrosine phosphatase (PTP) activity, thereby attenuating proliferation and migration of rat aortic SMC in vitro and reducing neointima formation following angioplasty in rat carotid arteries [69]. This implies that PTP modulation by ROS may augment PDGF-BB signalling which would consequently further augment the contribution of adventitial myofibroblasts to vascular remodelling. Considering the extensive evidence that oxidative modifications are intimately involved in vascular diseases and the controversy surrounding the limited benefits of dietary antioxidant interventions in human studies [85], in the context of adventitial oxidative stress, an alternative therapeutic strategy may be to locally transfer protective endogenous antioxidant genes to modulate perivascular redox status. Gene transfer studies by direct vector injection to the vessel wall of adenoviruses encoding superoxide dismutase and catalase have been shown to improve endothelial function, reduce ROS generation and restenosis following iliac artery angioplasty in a rabbit model of atherosclerosis [86]. This was likely to have counteracted
the decreased arterial activity of SOD observed to be associated with constrictive remodelling in a rabbit model of vascular injury [87]. Recently Levonen et al. [88] reported that luminal gene transfer with an adenoviral vector coordinating expression of the transcription factor nuclear factor E2-related factor (Nr2f2) reduces oxidative stress and inflammation in the vessel wall at 14 days following balloon angioplasty in rabbit aortas. Nr2f2 is the key transcription factor that regulates concerted induction of several antioxidant enzymes such as heme oxygenase-1 (HO-1) [89,90], which exhibits anti-atherogenic properties by virtue of its key role in catalysing the pro-oxidant heme to generate the vasodilator carbon monoxide (CO) and lipid peroxide scavenging antioxidant biliverdin [91,92]. In the context of vascular remodelling, local adenoviral HO-1 gene transfer or administration of CO or biliverdin have been shown to reduce neointimal hyperplasia following experimental vascular balloon injury or vein graft arterialisation in rats [93–95]. Interestingly, the anti-restenotic properties of the antioxidant drug probucol have also been attributed to vascular HO-1 induction following endothelial denudation of rabbit aortas [96]. In addition, it is conceivable that enhanced adventitial ROS generation may further act to augment differentiation of progenitor cells toward a myofibroblastic phenotype which can contribute to neointimal hyperplasia [97]. Taken together, it is likely that further research to evaluate perivascular gene transfer to antagonise growth factor signalling, inhibit NAD(P)H oxidase activation and enhance expression of endogenous antioxidant defence genes will yield novel therapeutic strategies to modulate redox signalling pathways that reduce adventitial activation and vascular remodelling in atherosclerosis and following angioplasty.

8. Summary and future perspectives

The potential benefits of vascular gene transfer as a research tool and potential therapeutic strategy for cardiovascular disease have been well documented over the past decade [98,99]. In particular, perivascular gene delivery from the ‘outside-in’ through the adventitia may be advantageous by virtue that it can be performed by direct application of vectors during surgical procedures that yields localised transgene expression in the adventitial compartment with fewer pro-inflammatory effects compared to intraluminal gene delivery [100,101,102]. Currently only about 5% of percutaneous interventional angioplasty procedures in developed countries are performed without stent implantation, however, although restenosis without stenting has become a relatively minor clinical problem, the pro-inflammatory mechanisms underlying in-stent restenosis as well as vein graft failure similarly involve enhanced growth factor signalling, ROS generation and stem cell differentiation leading to adventitial cell activation and neointimal hyperplasia [103–105]. However, for successful clinical implementation of perivascular gene therapy, larger trials remain to be performed and long term safety and efficacy considerations of utilising a variety of viral and non-viral therapeutic vectors must be further addressed for in-stent restenosis and vein graft failure [106,107]. The biological activity of overexpressed transgenes delivered to the vessel wall should also be treated with caution in the context of the disease model. For example, eNOS gene delivery to the adventitia [71] may result in uncoupling of the enzyme to generate deleterious superoxide under certain conditions [107]. Furthermore, strategies to modulate perivascular TGF-β1 activity [24,26,37] may lead to advanced atherosclerotic plaque destabilisation [108], and vascular VEGF gene transfer [52] may lead to plaque neovascularisation, haemorrhage and expansion [109]. Nevertheless, this review has highlighted the promise of efficient adventitial gene delivery to modulate fibroblast and progenitor cell growth factor and redox signalling pathways as an important experimental tool to provide further mechanistic insights for clinical perivascular therapy approaches to reduce the incidence of restenosis following angioplasty, stent implantation and coronary artery bypass grafting. It is possible that gene delivery to the adventitia following angioplasty and bypass procedures through use of coated drug-eluting perivascular stent cuffs [110,111] may represent a future clinical strategy for the reduction in the incidence of restenosis and vein graft failure.

Acknowledgements

Studies performed by the authors as cited in this review were supported by the British Heart Foundation. Dr Adrian Churchman is a British Heart Foundation funded postdoctoral research associate. We are very grateful to Professor Giovanni Mann, Cardiovascular Division, King’s College London, for helpful scientific discussion and encouragement during the preparation of this review.

References


[77] Leite PF, Danilovic A, Moriel P, Dantas K, Marklund S, Dantas AP, et al. Sustained decrease in superoxide dismutase activity underlies constric-


