Mechanisms of Vein Graft Adaptation to the Arterial Circulation – Insights Into the Neointimal Algorithm and Management Strategies –

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For patients with coronary artery disease or limb ischemia, placement of a vein graft as a conduit for a bypass is an important and generally durable strategy among the options for arterial reconstructive surgery. Vein grafts adapt to the arterial environment, and the limited formation of intimal hyperplasia in the vein graft wall is thought to be an important component of successful vein graft adaptation. However, it is also known that abnormal, or uncontrolled, adaptation may lead to abnormal vessel wall remodeling with excessive neointimal hyperplasia, and ultimately vein graft failure and clinical complications. Therefore, understanding the venous-specific pathological and molecular mechanisms of vein graft adaptation are important for clinical vein graft management. Of particular importance, it is currently unknown whether there exist several specific distinct molecular differences in the venous mechanisms of adaptation that are distinct from arterial post-injury responses; in particular, the participation of the venous determinant Eph-B4 and the vascular protective molecule Nogo-B may be involved in mechanisms of vessel remodeling specific to the vein. This review describes (1) venous biology from embryonic development to the mature quiescent state, (2) sequential pathologies of vein graft neointima formation, and (3) novel candidates for strategies of vein graft management. Scientific inquiry into venous-specific adaptation mechanisms will ultimately provide improvements in vein graft clinical outcomes. (Circ J 2010; 74: 1501–1512)

Key Words: Adaptation; Eph-B4; Nogo-B; Vein grafts; Veins

Vein is the gold standard for vascular graft conduits, particularly in the setting of arterial reconstructive surgery for patients with coronary artery disease and limb ischemia. Compared with other types of graft conduits, the most distinctive property of the vein graft is the adaptive response to the arterial environment during the post-surgical process; this adaptation is thought to be responsible for the superior performance of vein grafts compared with prosthetic grafts. Vein graft adaptation, commonly known as “arterialization”, is characterized by the process known as intimal hyperplasia; that is, vessel wall thickening with deposition of smooth muscle cells (SMC) and extracellular matrix (ECM) in all layers of the vein graft, but especially in the intima. Vascular remodeling in vein grafts is thought to be a normal and necessary response for environmental adaptation (eg, to the high wall shear stress and stretch force). However, it is also well-known that current reports show 20–50% of vein grafts are eventually complicated by remodeling, culminating in clinical sequelae to the patient (ie, vein graft thrombosis and failure).1,2

Because vein graft adaptation presents similar physiologic characteristics and clinical sequelae when compared to the post-arterial injury response, it has been hypothesized that they share the same underlying mechanism. Even the term “neointimal hyperplasia”, usually applied to pathological remodeling after arterial injury, has been extended to pathological excessive venous remodeling. This observation has lead to a number of current therapies in the post-vein graft surgery patient that are identical to those in the arterial injury patient, though clinical trials have not borne out the efficacy of these management techniques in the treatment of vein graft complications.3,4 Thus, it is likely that vein graft adaptation has specific and distinct differences in its molecular mechanisms (eg, those specific to venous cells) compared with the processes involved in arterial vessel wall remodeling after arterial injury. This suggestion is not yet fully understood, unfortunately, but some recent experimental investigations are beginning to clarify these differences. The purpose of this...
Figure 1. Mechanisms of arterial and venous determination during development of the embryonic vasculature. (A) In arterial endothelial cells, Sonic Hedgehog induces vascular endothelial growth factor (VEGF), which in turn activates Delta-Notch signaling. This cascade induces expression of the arterial-specific marker Ephrin-B2, and simultaneously limits expression of the venous-specific marker Eph-B4. (B) In venous endothelial cells, COUP-TFII actively blocks expression of the VEGF receptor, and subsequently expression of the Delta-Notch cascade. The failure of this cascade prevents expression of Ephrin-B2 while simultaneously stimulates Eph-B4 expression.

Figure 2. Morphologic and physiologic features of an artery and a vein. IEL, internal elastic lamina; EEL, external elastic lamina.
review is to highlight the underlying physiological and molecular events involved in vein graft adaptation, and to highlight recent propositions in the treatment of vein graft wall thickening.

Vein Wall Biology
In this section we draw attention to aspects of normal vein physiology in order to provide a foundation for understanding vein graft adaptation to the arterial environment. Despite the common basic function of arteries and veins in the transportation of blood, there are many differences in their organic background.

Development
Until the crucial role of transcriptional factor COUP-TFI in embryonic venous development was discovered in 2005, vein was thought to be the default vascular state from which undifferentiated vascular tissue developed, because few distinctive vascular markers had been identified that are specific to the venous lineage. It was known that in the specification stage of embryonic vasculature development, the Sonic Hedgehog–vascular endothelial growth factor (VEGF) cascade, located in arterial endothelial cells (EC), activated the Delta-Notch signaling cascade, which in turn induces expression of Ephrin-B2, a distinctive marker of arterial cell fate, and limits expression of Eph-B4, a known venous endothelial marker (Figure 1A). This cascade was found to be actively blocked by COUP-TFI in the development of veins, inhibiting the VEGF receptor at the initial venous development step. Blockade induces the failure of Delta-Notch activation and Ephrin-B2 expression in venous EC while simultaneously increases Eph-B4 expression (Figure 1B). These findings established that environmental factors are not solely responsible for the critical decision of vascular specificity; specific molecular mechanisms are responsible as well.

Morphology and Physiology
The structure of a normal vein wall mimics that of artery, as both vessel walls are composed of 3 distinct layers: the intima, the media, and the adventitia; these 3 layers are separated by the internal and external elastic layers (Figure 2). However, in the vein, cellular and fibrous components are significantly limited in number, particularly in the medial and elastic layers, leading to a vessel wall that is generally thinner than a comparable anatomic artery. Eph-B4 contributes significantly to this difference in wall thickness, as Eph-B4 limits mural cell recruitment, cell proliferation by ERK dephosphorylation, and vein wall thickness in mature tissue. Additionally, veins have a distinctive structure in their lumen: the valve (Figure 2 Right). Supporting blood return from peripheral tissues to the cardiac pump, the venous valves act to avoid blood reflux in their normal role. However, their presence contributes to impedance of laminar flow through the vein, and to an even greater degree under the increased flow and pressure of the vein graft in the arterial environment. This native disruption in the otherwise cylindrical vascular lumen causes a considerable turbulence in the blood flow that is known to induce vascular endothelial injury, and is thought to be a part of the intimal hyperplasia-forming mechanism in arterial interposed vein grafts.

In their normal physiological environment, the anatomical structure of the vein undergoes constant adaptation to flow and volume (Figure 2). The venous wall is usually exposed to a low pressure and low flow state; as a consequence, its structure requires high adaptability for a constantly changing volume load. The compliance of the thin venous wall supports this adaptive flexibility to variable local blood volume. Empirically, it is observed that despite a severe disruption of the normal venous blood environment, the vein easily adapts to its new state via a transition of the venous wall structure, as seen in the setting of an arterial vein graft, arteriovenous fistula or varicose vein. These findings further suggest that the venous structure is actively controlled by molecular elements, as well as physiological environments.

Quiescent Venous State and Important Molecules
The main cellular components of a vein wall in its normal state are EC and SMC. ECs form a flattened monolayer on the elastic basement membrane, and are thought to play an essential role in venous wall integrity and function. It is well known that healthy ECs produce the vasorelaxant prostacyclin (also known as PGI2), as well as endothelium-derived nitric oxide (NO), PGI2, produced primarily by cyclooxygenase, and NO, produced by NO synthase (NOS), work in coordinated fashion to prevent platelet activation, adhesion, and aggregation. NO is also known to be a negative conductor for the expression of chemical mediator secretion and inflammatory cell adhesion molecules (ICAM), ICAM-1 and VCAM-1. These normal EC functions exert an important force in the initial steps of vein graft neointimal formation, as we describe later.

SMCs also play an important role in vessel wall homeostasis. The interaction between SMCs and the ECM promotes a quiescent state in the SMCs in the normal venous environment via transforming growth factor (TGF)-β, heparin, and heparin-like-molecules. TGF-β negatively regulates SMC mitogenesis, and stabilizes the ECM, which works as a SMC migration “seawall”. Heparin is thought to be a neutralizing factor for fibroblast growth factor (FGF), and downregulates cell proliferation. This evidence shows that a quiescent vascular wall state is actively kept by endogenous molecules.

The normal vessel wall is characterized by a low rate of cell turnover. In particular, there are low rates of both cell proliferation and apoptosis. The low rate of cell turnover may promote mechanical vessel integrity during normal environmental conditions; however, after injury, or after a change in flow conditions, vessels can increase their rates of proliferation and apoptosis, reacting to the stimulus.

Algorithm of Pathological Vein Graft Wall Thickening
In 2005, Mittra et al reviewed the chronological events in vein graft neointima formation and broadly classified the events leading to a neointima into 5 steps: (1) platelet activation and correlated events; (2) inflammation with leukocyte recruitment; (3) activation of the coagulation cascade; (4) SMC migration and (5) proliferation. In this section, we describe the pathological algorithm of vein graft adaptation sequentially with the mechanisms and molecular interactions (Figure 3).

Surgical Injury
The etiology for all vein graft adaptation is initiated by surgical resection of the vein. The vein is likely to be in a quiescent state prior to surgical harvest, during which the adventitia is damaged when it is disrupted from its blood supply, the vasa vasorum. The consequences of this disruption include tissue hypoxia, as well as hyponutrition of the vessel wall. Vein graft hypoxia leads to the release of the inflam-
matory cytokines, which have a downstream impact on neointimal hyperplasia; interleukin (IL)-6 and IL-8 have been shown to be specifically released in this setting, but other known acute-phase reactants have yet to be demonstrated. It has been suggested that the degree of surgical graft adventitial injury is thus directly related to subsequent graft neointimal formation. To avoid adventitial injury, in situ vein graft conduits have been preferentially selected for vascular reconstruction in limb ischemia patients at some institutes.

**Pulsatile Wall Stretch and Hydrostatic Force**

When transplanted into an arterial environment, venous tissue is immediately exposed to intense pulsatile stretch forces and wall shear stress. It is known that wall stretch force induces cell apoptosis with subsequent cell proliferation. In some experimental models that supported the vein graft with a vascular external sheath, graft failure was prevented because of reduced direct wall stretch force. Data from these physiologic models are supported by reports of several molecular interactions induced by this environmental transition. Insulin-like growth factor-1 receptor (IGF-1R) and the tyrosine phosphorylation of its substrate IRS-1 are induced by mechanical stretch, activated Src, which is also induced by the same mechanical stretch, and autocrine IGF-1 production. Likewise, SMC derived from human saphenous vein demonstrates ERK phosphorylation, Akt/PKB phosphorylation, and Rho/Rho-kinase activation under cyclic stretch stimulation. These intracellular signaling cascades are known to induce cell phenotypic changes, inducing a transition from the quiescent, or contractile, phenotype, into a synthetic, migratory and proliferative phenotype, and also inducing apoptosis, as we describe later.

It is also known that the transition to an arterial environment with its higher wall shear “hydrostatic” stress injures the vein graft endothelium. In early stages of vein graft adaptation, physiological low-concentration NO is known to have an important role in preserving venous identity, and protecting the vascular wall from platelet-derived vasoactive substances and SMC relaxation, as well as inflammatory responses. Physiological NO is created by healthy ECs via endothelial NOS (eNOS) at venous levels of shear stress. Under arterial conditions, in which shear stress is comparatively much higher, the venous tissue homeostasis that induced NO production is disrupted because of EC injury.

The function of NO in limiting vein graft neointimal volume has been confirmed in the rabbit constitutive eNOS overexpression experimental model. On the other hand, highly concentrated pathogenic NO, produced by inducible NOS (iNOS) activation, is known to promote vascular injury and apoptosis via superoxide and peroxynitrite production, and subsequently increases vein graft neointimal volume. These findings have led to therapeutic experiments with NO manipulation. Recently, the effect of the addition of NO-donating aspirin (NO-ASA) was examined in a pig vein graft model. The negative neointimal regulation effect of NO-ASA was also confirmed in a human saphenous ex vivo experimental model that elicited venous tissue relaxation, cGMP promotion, and inhibition of SMC proliferation. Because gastrointestinal, antithrombotic, and antiatherogenic effects were observed in the NO-ASA experiments, it is thought that this is one of the NO donating applications that is closest to approaching clinical trials.

**Platelet Adhesion, Aggregation, and Activation**

An injured endothelium quite rapidly acts as a theater for platelet events, within 1 min of vein graft implantation. At the site of endothelial injury, exposed subendothelial matrix following injured endothelial denudation leads to the adherence and aggregation of platelets. Numerous cytokines and other bioactive substances are released by activated platelets; for instance, adenosine diphosphate is known to be released from adhered platelets, and it activates the arachidonic acid synthesis pathway that produces thromboxane A2, a potent chemo-attractant and a SMC mitogen. Several other growth factors and cytokines, such as platelet-derived growth factor (PDGF), TGF-β, IL-1, IL-6, IL-8, and thrombin, are also known to come into play following these events. Therefore, platelet aggregation is thought to be a target for limiting vein graft wall thickening. In a recent report, cilostazol, an inhibitor of cyclic adenosine monophosphate phosphodiesterase III (PDE-III inhibitor), was proposed to have a protective effect for neointimal hyperplasia formation in the rat VG model.

**Leukocyte Recruitment and Inflammation**

During the subsequent week after implantation, leukocyte recruitment and the inflammatory response play the primary roles in vein graft adaptation. Chronic inflammation in the
graft wall is initiated by adherence molecule expression following platelet activation at the site of the injured endothelium. Leukocytes attach and roll on the endothelial surface while undergoing activation via the interaction of leukocyte surface P-selectin glycoprotein ligand (PSGL)-1 and P-selectin on the bound adherent platelet.\(^\text{44,45}\) Activated leukocytes then migrate into the graft vessel's wall, an action mediated by adherence molecules such as monocyte chemotactic protein (MCP)-1,\(^\text{46}\) ICAM,\(^\text{47,48}\) Mac-1,\(^\text{49}\) and GPlbα.\(^\text{50}\) Although TGF-β overexpression is sustained until the late phase of vein graft adaptation, MCP-1 works primarily during the early phase of vein graft adaptation and diminishes after 1 week, as observed in an experimental animal model.\(^\text{51}\) These findings support the idea that adherence molecule expression and leukocyte recruitment interact significantly, and that these events are essential in the early phase of graft adaptation.

It is known that the degree of leukocyte migration is an important factor in the level of neointimal thickening that a vein graft develops. Peppel et al showed that 60% of the neointimal area in the ROSA26 mice vein graft model was derived from cells extrinsic to the graft.\(^\text{52}\) They also found that green fluorescent protein (GFP)-expressing bone marrow cells transplanted into the C57Bl/6 mouse recipient showed abundant GFP-positive cells in vein graft neointima. Thus, leukocyte migration is thought to be a noteworthy target for vein graft neointimal hyperplasia treatment. Experimentally, by blocking the MCP-1 receptor, CCR-2, using 7N-D-MCP-1 gene transduction in an ApoE3Leiden mouse vein graft model, a 51% volume reduction in neointimal hyperplasia was observed.\(^\text{46}\)

Similarly, recruitment of the surrounding cells and adventitial inflammation also play important roles in neointimal development.\(^\text{53}\) Adherence molecule E-selectin has been shown to be induced in the post-injury adventitial layer, and furthermore, mediates subsequent inflammation.\(^\text{53}\) Recognition of E-selectin by the cells surrounding the vein graft is thought to further contribute to vein graft adaptation; in the ROSA26 transgenic mouse model, isolation of the graft from the surrounding cells resulted in a 90% reduction in the number of SMCs in the vein graft neointima.\(^\text{52}\) Although adherence molecules have been shown to strongly contribute to neointimal hyperplasia, growth factors PDGF-BB and TGF-β are also known to influence local adventitial cell accumulation, angiogenesis, ECM deposition, and enhanced generation of reactive oxygen species, and therefore subsequently contribute to neointimal hyperplasia volume.\(^\text{54}\)

Leukocyte recruitment is accelerated by immune responsive substances secreted from graft wall component cells and the leukocytes themselves. IL-8 is a known neutrophil chemoattractant. IL-1, IL-6 and tumor necrosis factor (TNF)-α, in association with reactive oxygen species, numerous growth factors, and proteolytic enzymes, modulate the inflammatory responses.\(^\text{55}\) These bio-attractant agents play an important role in the late phase of vein graft neointimal formation following adherence molecule expression.\(^\text{56}\) The degree of wall shear stress is also known to be a trigger for cytokine secretion. A low shear stress model of vein graft adaptation was observed to develop an increased intimal volume because of the secretion of the pro-inflammatory cytokine IL-1β, whereas a high shear stress model was found to limit wall thickening via the secretion of the anti-inflammatory cytokine IL-10.\(^\text{56}\)

Given the role of inflammation in the formation of the neointima, suppression of the immune reaction is a theoretical option in the treatment of pathologies associated with neointimal hyperplasia. For example, the vaccinia virus protein 35K, which possesses an antiinflammatory effect, was analyzed in ex vivo adenoviral transfection and was discovered to reduce rabbit vein graft neointimal hyperplasia by limiting macrophage infiltration.\(^\text{57}\) Flavonoid, which is known to have antiinflammatory, antiallergic, and antioxidant effects, also attenuates rat vein graft neointima formation through the downregulation of PDGF-BB and TNF-α.\(^\text{58}\) Another immunosuppressive agent, FK778, is known to limit neointimal hyperplasia in the rat VG model.\(^\text{59}\)

Rapamycin, the most well analyzed immunosuppressive agent, acts via mTOR inhibition in the Akt signaling pathway, and is anticipated to have an antiproliferative effect clinically. Experimentally, rapamycin has been shown to inhibit lipopolysaccharide-dependent TNF-α release from human saphenous-vein-derived SMC in culture.\(^\text{60}\) In vivo, perivascular rapamycin application by pluronic gel shows a dose-dependent reduction of neointimal hyperplasia because of decreases inflammatory cell infiltration and increased cell apoptosis.\(^\text{61,62}\)

The inflammatory cytokine, TNF, has a unique mechanism for vein graft neointimal regulation: it regulates SMC mitogenesis via complementary costimulation with other growth factors during vein graft intimal hyperplasia formation.\(^\text{63}\) In a genetic knockout experimental model, TNF receptor-1 amplifies neointimal hyperplasia volume and upregulates leukocyte adherence molecules such as MCP-1.\(^\text{64}\) On the other hand, TNF signaling via TNF receptor-2 reduces vein graft neointimal hyperplasia by suppressing the adhesion molecule V CAM-1 and attenuating EC apoptosis.\(^\text{65}\)

**Coagulation Cascade and Thrombosis**

Endothelial injury also activates the coagulation cascade. Thrombus formation at the site of injured vein graft endothelium is well known to contribute not only to promotion of acute-phase reactants but also to intimal hyperplasia formation in the vein graft. Tissue factor (TF) is the key mediator of coagulation at the site of vessel wall injury when the subendothelium is exposed to the circulating blood.\(^\text{14}\) TF, a glycoprotein found in vessel walls and mononuclear cells, binds to coagulation factor VII/VIIa to initiate the coagulation cascade. Downstream from VII/VIIa activation, release of coagulation factor Xa and PDGF from activated platelets plays a role in SMC mitogenesis.\(^\text{56}\) Circulating TF is incorporated into the forming thrombus and activates platelets, which accelerates thrombin generation, and is additionally a known SMC proliferation agonist.\(^\text{67,68}\) Thus, studies of inhibition of TF function have been undertaken in a rabbit model, and these have demonstrated limitation of vein graft neointimal formation.\(^\text{69}\)

Because vessel wall thrombus induces SMC proliferation, it is thought that anticoagulant drugs may have potential vein graft wall-limiting effects. For example, local aspirin application in a mouse vein graft model attenuated acute-phase graft thrombosis.\(^\text{70}\) However, on examination of human coronary grafts, aspirin did not reduce the incidence of long-term vein graft failure.\(^\text{71}\) Likewise, anticoagulant proteins, such as thrombomodulin, and the EC protein C receptor have been shown to reduce early stage vein graft thrombosis, but they do not contribute to long-term prevention of neointimal hyperplasia.\(^\text{72}\) Another anticoagulant, tissue plasminogen activator (tPA), is described as a regulator of ECM remodeling in vein grafts.\(^\text{73}\) In an animal model, perivascular treatment with tPA modified the ECM gradients and prevented neointimal hyperplasia by altering SMC migration.

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*Mechanisms of Vein Graft Adaptation* 1505

_Circulation Journal _ Vol.74, August 2010
Role of Growth Factors in SMC Migration and Proliferation

The common result of the multiple forms of vascular injury as described is an alteration in the character of the SMCs in the vessel wall. By 1–4 weeks after vein graft implantation, the SMCs change their phenotype from the quiescent contractile state to the synthetic motile state, and migrate into the neointima from the medial layer.14 Synthetic-state SMCs subsequently undergo rapid proliferation and growth to form the neointimal lesion. Simultaneously, vascular injury itself is known to accelerate cell apoptosis in the neointimal layer. In this mitogenic, synthetic, and apoptotic complex state, numerous extracellular stimuli and intracellular signal transduction cascades are presumed to be involved.

In animal models, several growth factors have been well described in the process of vein graft neointimal formation: TGF-β, VEGF, basic-FGF (bFGF), insulin-like growth factor (IGF)-1, and PDGF. The majority of growth factor receptors are cell membrane-type tyrosine kinases that trigger downstream signal cascades, including the mitogen-activating protein kinase (MAPK) pathway and phosphatidyl-inositol 3 kinase (PI3K)–Akt/PKB pathway. These pathways involve proliferation, survival, differentiation and migration of SMCs, so growth factors are thought to be effective targets for controlling the thickening of the vein graft wall.

VEGF is known to be a negative regulator of neointimal formation in vein graft animal models.15-17 The mechanism for this regulation is thought to be accelerated re-endothelialization of the injured vessel, increased NO production,18 and communication with other growth factor pathways. VEGF is also known to be a key initiator molecule for vascular determination.19,20 Thus, VEGF manipulation in vein grafts may protect venous-specific biology during adaptation. IGF-1 is known to play a pivotal role in SMC proliferation in the vascular system. In venous SMC, stimulation of the stretch force induces expression of IGF-1 and its receptor IGF-1R, and activates downstream intracellular signaling cascades that are both IGF-1 dependent and independent.21 Because the IGF-1–PI3K–Akt/PKB cascade regulates SMC cycle progression,22,23 therefore, it may be an effective target of pharmacological manipulation for management of vein graft failure.

TGF-β2 is often described as a positive regulator of neointimal formation, consistent with its role in the formation and stabilization of the ECM. Antisense (blocking) TGF-β1 mRNA prevented collagen accumulation in a rat vein graft model by reducing TIMP-1 (tissue inhibitor of metalloproteinase (MMP)-1) and collagen expression, and changing the proportions of the SMC and immune cell populations.24 Ex vivo organ culture model of the human saphenous vein also demonstrates that a TGF-β1 antagonist reduces collagen content and the degree of intimal hyperplasia.25 Contrarily, Activin-A, which is also in the TGF-β super family, induces low SMC proliferation rates and reduces neointimal hyperplasia in rat vein grafts.26 bFGF also effects positive regulation during vein graft neointimal formation. In a rabbit vein graft model, antisense bFGF coding adenovirus limited neointimal hyperplasia by an ERK-dependent mechanism.27 PDGF also has been shown to accelerate SMC proliferation in both human arteries and veins.28 Positive regulation of SMC proliferation by PDGF has been confirmed in vein graft models. Blockade of PDGF–MAPK–AP1 signaling attenuates neointimal formation in mouse vein grafts.29 and PDGF-R inhibitor reduced cell proliferation and subsequent neointimal hyperplasia in a rabbit model.30 Midkine, which is a heparin-binding growth factor, has been recently described as a further positive regulator of neointimal hyperplasia via its role in inflammation and cell proliferation; siRNA blockade of its activity has been shown to reduce neointimal hyperplasia in an animal model.31

Intracellular Signaling

Several cytokines and growth factors are involved in varied intracellular signaling cascades during vein graft adaptation, so they are commonly proposed as molecules for potential targeted therapy of neointimal hyperplasia. The MAPK signaling transduction pathway is a well-known part of the cellular mitogenic responses, and demonstrates an important role during vein graft adaptation. A canine vein graft model showed that ERK1/2, a key molecule in the MAPK cascade, accelerates medial-layer cell proliferation, suppresses apoptosis, and induces inflammatory cell infiltration.32 The MAPK signaling cascade is also known to control MMPs and TIMP-2 expression in vein grafts.33

The PI3K–Akt/PKB pathway is also important in cell proliferation and additionally contributes to cell survival in the vein graft neointima. Modulation of PI3K signaling through PTEN, which is a downstream regulator of PI3K, prevents PDGF-dependent Akt/PKB phosphorylation and limits intimal hyperplasia by decreasing SMC proliferation.34 PI3K–Akt/PKB is also known to induce p53, a pro-apoptotic transcriptional factor, and thus accelerate apoptosis.35,36 In a porcine vein graft model, overexpression of p53 induced apoptosis of SMCs, resulting in improvement of the graft’s lumen diameter at medium-term follow-up.37 Perivascular treatment with rapamycin also accelerates apoptosis in mouse vein grafts, because of PI3K–Akt/PKB signal activation via mTOR inhibition.38 Additionally, in vitro, SMCs derived from the human saphenous vein show higher Akt/PKB phosphorylation rates than arterial SMCs.39 These findings support Akt’s importance in cell survival during venous neointimal formation.

Small guanosine triphosphate-binding proteins (small G protein) have an important role in cytoskeletal dynamics, which are a vital component of cell migration. Statins, commonly used clinically for serum lipid reduction as well as plaque stabilization, are HMG-CoA reductase inhibitors and additionally have vascular protective effects through inhibition of Rho and Rho-associated kinase activity. As described previously, Rho/Rho-kinase are induced under pulsatile stretch conditions in venous tissue, and statins specifically inhibit this sequence and thus reduce SMC proliferation.40 Limitation of Rho-kinase activity increases eNOS expression as well, and it is thought to be the part of the mechanism that limits neointimal hyperplasia.41,42 Furthermore, simvas-tatin downregulates the RhoA/ROCK pathway, and reduces MMP-9 secretion.43 In 2005, Komori et al found that pravas-tatin treatment limits growth factor production and neointimal hyperplasia in a rabbit vein graft model.44,45

Transcription Factors

Vein graft implantation results in a hyperproliferative state in which stimulation of growth factors induces accelerated activation of transcription factors and subsequent cell cycle entry and protein turnover. Therefore, numerous transcription factors have been investigated as targets for neointimal hyperplasia prevention and treatment. For instance, Rb (retinoblastoma) protein, which inhibits E2F, showed significant reduction of neointimal hyperplasia formation in a human saphenous vein organ culture model.46 E2F is the first transcription factor targeted in humans to prevent vein graft neo-
intimal proliferation.\textsuperscript{99} Ex vivo intravascular application of decoy-oligodeoxynucleotide (ODN) to vein grafts was implemented prior to implantation. Although late-phase clinical trials resulted in no significant improvement in graft patency in the experimental group in either the coronary or peripheral vein grafts,\textsuperscript{3,4} this trial has advanced the possibilities of vein graft management.

In the human ex-vivo organ culture model, angiotensin II induces MAPK, which activates transcription factor activator protein (AP)-1. AP-1 increases connexin-43 expression, which

\begin{table}[h]
\centering
\caption{Experimental Manipulation of Vein Graft Neointimal Hyperplasia}
\begin{tabular}{|l|l|l|l|l|}
\hline
Mechanism & Manipulated factor & Vein graft model & Reference no. \\
\hline
EC damage, platelet aggregation, and thrombosis & & & \\
\hline
eNOS & eNOS gene & Rabbit & 36 \\
Superoxide & Celiprolol & Rabbit & 37 \\
NO & NO-donating aspirin & Pig & 38 \\
PDE-III & Citostazol & Rat & 43 \\
Tissue factor & rTFPI* & Rabbit & 69 \\
Thrombosis, EC damage & Aspirin & Mouse & 70 \\
Tissue plasminogen activator microspheres releasing & IPA & Rabbit & 73 \\
\hline
Immunoresponse & & & \\
\hline
MCP-1 & 7ND-MCP-1 & Mouse & 46 \\
ICAM-1 & ICAM-1 knockout mouse & Mouse & 48 \\
TGF-\textalpha, MCP-1 (monocyte accumulation) & Lip-Clod** & Rat & 50 \\
IL-6, IL-8 & N-acetylcysteine & Rat & 25 \\
Chemokine & Vaccinia virus protein 35K & Rabbit & 57 \\
Immunoresponse & FK778 & Rat & 59 \\
TNF & TNF receptor knockout mouse & Mouse & 64, 65 \\
TNF-\textalpha, PDGF-BB & Flavonoid & Rat & 58 \\
\hline
Growth factors & & & \\
\hline
VEGF & Recombinant VEGF & Rabbit & 74 \\
VEGF-Eph-B4 & VEGF siRNA & Rat & 10 \\
TGF-\beta & Anti-sense bFGF & Rat & 80 \\
TGF-\beta & Activin-A & Rat & 82 \\
bFGF & Antisense bFGF & Rabbit & 83 \\
PDGF & Suramin & Mouse & 85 \\
PDGF receptor & Imatinib mesylate & Rabbit & 86 \\
Midkine & Midkine siRNA & Rabbit & 87 \\
\hline
Intracellular signalings & & & \\
\hline
ERK-1/2 & ERK-1/2 inhibitor & Dog & 88, 89 \\
mTOR & Rapamycin & Mouse & 61, 62 \\
PTEN & PTEN adenovirus & Dog & 90 \\
Rho & Statin & Rabbit & 94, 97 \\
\hline
Transcriptional factors & & & \\
\hline
p53 & p53 adenovirus & Pig & 91 \\
p53 & p53 knockout mouse & Mouse & 92 \\
AP-1 & AP-1 decoy ODN & Rabbit & 102 \\
cJun & cJun DNAzyme & Rabbit & 104 \\
NF\textalpha-B & NF\textalpha-B decoy ODN & Rabbit & 105 \\
NF\textbeta-B & NF\textbeta-B decoy ODN & Dog & 106 \\
\hline
Extracellular matrix deposition & & & \\
\hline
MMPs & TIMP-1 adenovirus & Rabbit & 57 \\
MMPs & TIMP-1 plasmid DNA & Mouse & 109 \\
MMPs & TIMP-2 adenovirus & Mouse & 111 \\
MMP-3 & MMP-3 adenovirus & Rabbit & 112 \\
\hline
Others & & & \\
\hline
Nogo-B & Nogo-B adenovirus & Pig & 114 \\
Endothelin & Endothelin-1A receptor antagonist & Pig & 103 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{*Recombinant human tissue factor pathway inhibitor; **liposomally encapsulated dichloromethylene bisphosphonate. EC, endothelial cell; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PDE, phosphodiesterase; MCP, monocyte chemotactic protein; ICAM, inflammatory cell adhesion molecule; TGF, transforming growth factor; IL, interleukin; VEGF, vascular endothelial growth factor; bFGF, basic-fibroblast growth factor; TNF, tumor necrosis factor; PDGF, platelet-derived growth factor; AP, activator protein; ODN, oligodeoxynucleotide; MMP, metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.}
positively regulates neointimal hyperplasia in saphenous veins via SMC proliferation and migration.\textsuperscript{100,101} Additionally, endothelin-1 triggers SMC proliferation and acts via endothelin A/B receptors. Decoy-ODN against AP-1 reduces neointimal hyperplasia in a rabbit vein graft model via this endothelin A/B receptor downregulation.\textsuperscript{102} Endothelin-1A receptor antagonist treatment also reduced neointimal hyperplasia in a pig vein graft model.\textsuperscript{103} Thus, AP-1 may be a future candidate for neointimal hyperplasia prevention.\textsuperscript{102}

Other transcription factors cause distortion of the vascular phenotype in experimental models. p53 null mice have increased neointimal hyperplasia lesions in vein grafts because of accelerated cell proliferation, migration, MMP expression, and reduced apoptosis.\textsuperscript{104} c-Jun, a shear-responsive transcription factor, induces SMC proliferation, MMP-2 secretion, and subsequently increases the neointima in explanted human saphenous vein and rabbit vein graft models.\textsuperscript{105} Nuclear factor (NF)-κ-B, which is a positive regulator of neointimal hyperplasia and is activated by TNF,\textsuperscript{106} is known to control cytokine and adhesion molecule gene expression. NF-κ-B treatment of vein grafts by decoy-ODN causes suppression of neointima formation in multiple animal models.\textsuperscript{107,108} Peroxisome proliferator activated receptor (PPAR)-γ is also described as a transcription factor with potential to limit neointimal hyperplasia, as it is involved with regulation of the cell cycle, cellular senescence, and apoptosis.\textsuperscript{109}

**ECM Deposition**

During vein graft adaptation, ECM degradation is a necessary process for SMC migration into the neointimal layer. MMPs are key enzymes in ECM degradation and remodeling in injured vessel walls, so inhibition of these molecules is another potential target for limiting vein graft neointima formation.\textsuperscript{110} For example, via alteration of the ECM components, MMP-9 induces the SMC proliferative phenotype and subsequently accelerates neointimal hyperplasia.\textsuperscript{109,110} c-Jun induces MMP-2, which also indirectly stimulates the SMC proliferative phenotype during vein graft adaptation.\textsuperscript{104} Furthermore, TIMP-2 treatment of vein grafts consistently attenuated neointimal formation in a mouse model.\textsuperscript{111} Interestingly, in vivo adenoviral MMP-3 transfection reduces rabbit vein graft stenosis and neointimal hyperplasia.\textsuperscript{112} Therefore, the functions of MMPs are complex, with overlapping or bidirectional interactions, and comprehensive clarification is necessary for true understanding of the specifics of MMP activity during vein graft wall remodeling.

**Novel Targets for Management of Vein Graft Thickening: Spotlights for the Future**

Although there are numerous potential experimental treatments for vein graft wall thickening that are successful in animal models, as described above (Table), no accepted strategy for management of vein graft neointimal hyperplasia has translated into successful clinical use for human patients. This section introduces novel concepts for vein graft neointimal hyperplasia management and cutting edge drug/gene delivery systems.

**Novel Molecular Mechanism Involved in Vein Graft Adaptation**

As described above, there are molecular differences in the development of the embryonic arterial and venous systems, including varied molecular determinant molecules.\textsuperscript{5} In 2007, Kudo et al described the involvement of the venous specific molecule Eph-B4 during vein graft adaptation.\textsuperscript{110} They reported that Eph-B4 is functional not only in embryonic development but also in mature, adult venous tissue, which suggests that the VEGF–Delta/Notch–Ephrin-B2/Eph-B4 cascade has some potential to regulate or induce a “venous specific” mechanism of neointimal hyperplasia.

Another molecule, Nogo-B, which was discovered in 2004 to be a molecule that limits arterial injury and thus is protective for the arterial wall,\textsuperscript{113} presents an interesting expression pattern in human and rat venous neointimal SMCs.\textsuperscript{114} Nogo-B expression is diminished in arteries after wire injury in mouse models; however, the vein graft neointima demonstrates Nogo-B upregulation, particularly in the SMC layer. Though Nogo-B expression in vein grafts and after arterial injury shows opposite patterns, overexpression is known to attenuate neointimal hyperplasia of both arterial injury sites and vein grafts.\textsuperscript{113,114} Because the specific mechanisms of Nogo-B function are not currently understood, it may be a formidable option for regulating neointimal formation. However, these findings support the hypothesis that there are different mechanisms involved in the formation of arterial and venous neointima.

**Gene/Drug Delivery for Vein Graft Management**

An important concept in the development of potential therapies for vein graft neointimal hyperplasia is the insight that the pathology is localized to the graft itself. As such, development of a local gene/drug delivery system would be a valuable tool, allowing increased local concentrations of drug delivery with minimal systemic side-effects. Direct ex vivo transfection or drug treatments have been used in the past for gene or drug delivery (ie, directly exposing the explanted vein to the drug on the back table of the operating room prior to surgical implantation of the treated vein).\textsuperscript{3,4,99} The advantage of this method is ease of application; however, the disadvantage is that treatment effects may be short and insufficient. Current animal models of vein grafts most commonly use local reagent delivery by perivascular application with pluronic gel\textsuperscript{10,43,61,62,70} or atelocollagen.\textsuperscript{67} Additionally, gelatine hydrogel drug wrapping for bFGF delivery was recently reported with good effect in a rat vein graft model.\textsuperscript{115} Nanoparticle- and microsphere-mediated drug delivery systems have also been reported in animal vein graft models.\textsuperscript{73,86} These slow release methods might be additional useful options for drug delivery direct to the graft.

The safety and efficacy of gene administration vectors is currently controversial. In most studies, replication-deficient recombinant adeno viral vectors are used for gene transfection in venous tissue. Adenoviral vectors require simple handling techniques, show easy viral concentration, and have the ability to transfect the gene of interest into quiescent cells. RGD-4C integrin-targeting-peptide inserted adenovirus is being developed to improve the efficiency of vascular EC- and SMC-specific gene delivery.\textsuperscript{116} The main disadvantage of adenoviral vector delivery is the transient nature of transfection. The transfected gene is not able to insert itself into the host genome and is thus diluted with cell proliferation. Adeno-associated virus has also been reported as a useful delivery vector, with reduced immunogenicity.\textsuperscript{117} Lentivirus vector, which is a member of the retrovirus vector family, has advantages over adenovirus vectors. Lentivirus provides not only high transfective efficiency into quiescent cells, but also permanent expression of the transfected gene, as the target gene is inserted into the host genomic DNA. Third-generation lentivirus, with an improved safety profile over
Mechanisms of Vein Graft Adaptation

earlier generation lentiviruses, has high transfection efficiency in human venous vascular cells,\textsuperscript{118} thus making it possibly the optimal tool for gene transduction into vein grafts.

**Conclusion**

Since Kunlin first described the use of autogenous veins as grafts in arterial repair in the early 1950s,\textsuperscript{119,120} patients with ischemic disease have received benefit from veins used as reconstructive conduits for arterial surgery. Over the past 60 years, numerous physiologic and molecular mechanisms of vein graft adaptation have been discovered. However, this complex process has yet to be characterized well, and we do not yet have the ability to therapeutically control graft wall thickening. The current progress in vascular biology is exciting; a recent example with potential therapeutic applications is the recently discovered microRNAs that are involved in the growth and differentiation of vascular SMCs.\textsuperscript{121} This development is attracting significant attention by researchers in the field of vascular biology,\textsuperscript{122,123} and their role in vein graft adaptation will likely be clarified in the near future.

For improved clinical outcomes of vein grafts, it may be necessary to regulate both negative and positive wall thickening. The proper balance of positive and negative remodeling may be one of the most difficult clinical problems in understanding vein graft adaptation, because it is critical to balance the outward adaptation necessary to carry arterial blood flow to the distal tissues with the inward forces preventing vein graft deterioration and failure. As such, we believe that study of the underlying venous-specific mechanisms of remodeling is critical. It is hoped that this scientific inquiry will be able to provide improved care and quality of life for patients with vascular disease.

**Acknowledgments**

This review study was supported in part by the National Institute of Health grant R01-HL095498-01, the American Vascular Association William J von Liebig Award, as well as by the resources and use of facilities at the VA Connecticut Healthcare System, West Haven, CT, USA.

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