Improving the Outcome of Vein Grafts: Should Vascular Surgeons Turn Veins into Arteries?

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Autogenous vein grafts remain the gold standard conduit for arterial bypass, particularly for the treatment of critical limb ischemia. Vein graft adaptation to the arterial environment, i.e., adequate dilation and wall thickening, contributes to the superior performance of vein grafts. However, abnormal venous wall remodeling with excessive neointimal hyperplasia commonly causes vein graft failure. Since the PREVENT trials failed to improve vein graft outcomes, new strategies focus on the adaptive response of the venous endothelial cells to the post-surgical arterial environment. Eph-B4, the determinant of venous endothelium during embryonic development, remains expressed and functional in adult venous tissue. After surgery, vein grafts lose their venous identity, with loss of Eph-B4 expression; however, arterial identity is not gained, consistent with loss of all vessel identity. In mouse vein grafts, stimulation of venous Eph-B4 signaling promotes retention of venous identity in endothelial cells and is associated with vein graft walls that are not thickened. Eph-B4 regulates downstream signaling pathways of relevance to vascular biology, including caveolin-1, Akt, and endothelial nitric oxide synthase (eNOS). Regulation of the Eph-B4 signaling pathway may be a novel therapeutic target to prevent vein graft failure.

Keywords: vein graft, ephrin-B2, Eph-B4, vessel identity

Introduction

Arterial stenoses and occlusions contribute to ischemic cardiovascular diseases, the leading cause of death worldwide. Bypass surgery using vein grafts as a conduit around these lesions has developed as the mainstay approach to reperfuse the ischemic organs and tissues ever since Kunlin first described the use of autogenous veins as grafts for arterial repair in the 1940s.1–5 Besides autologous veins, numerous alternative prosthetics such as Dacron and polytetrafluoroethylene have been developed and used as alternative conduits when vein grafts are not available. However, the mid- and long-term patency rates of prosthetic grafts are inferior to autogenous vein grafts, and therefore, autogenous saphenous vein grafts remain the gold standard for bypass surgery, particularly in the treatment of critical limb ischemia.6–8

During surgical creation of the vein bypass, the saphenous vein is separated from its physiological environment, necessarily inducing injury.9,10 After harvest of a reversed vein graft, the vein loses blood flow and pressure within its lumen, and then typically is given a stretch injury as it is reperfused. Marking dye is often used on the outer surface to prevent twisting of the vein, which injures the vein wall and is associated with altered venous cell migration and proliferation.1,11,12 In situ vein grafts require valve destruction with a valvulotome, directly producing intimal injury. Most importantly, the implanted vein is then exposed to arterial flow, with pressure, shear stress, and oxygen content distinctly different from that within the venous environment, effectively producing an injury similar to an ischemia-reperfusion mechanism. The vein graft responds to the surgical injury and arterial environment by integrating these multiphasic stimuli, typically resulting in favorable adaptation; however, in 20–30% of cases the vein graft cannot adapt successfully, with poor clinical consequences (Fig. 1).
Peripheral artery bypass failure can be classified into early, intermediate, and late failure. Early failure occurs within 6 months after implantation, and most commonly within one month, mainly due to technical factors, hypercoagulability, and compliance mismatch. Intermediate failure occurs between 6–24 months after surgery and is mainly caused by neointimal hyperplasia (NIH); it is the most common etiology of vein graft failure. Late failure generally occurs after 24 months and is typically associated with progressive atherosclerosis (Fig. 1). To reduce the incidence of vein graft failure and thus achieve improved long-term outcomes after vein graft implantation, we need to understand the vein graft’s response to the arterial environment.

**Vein Graft Adaptation**

In the first report of vein graft bypass for arterial repair, Kunlin observed venous wall thickening after an initial phase of progressive dilation, calling this adaptive process “arterialization.” Dilation of vein grafts is described as a shear-dependent early response that occurs in the first month after implantation; the endothelial cells are critically important in transducing the shear stress signal to the rest of the vessel wall. The thickening of the vein graft wall is characterized by accumulation of smooth muscle cells (SMC) and extracellular matrix components, similar in mechanism to the neointimal hyperplasia that forms after injury of arterial intima. Outward remodeling, e.g., increased diameter, and wall thickening are considered to be essential in clinically successful human vein grafts.

It is likely that all arterial bypass grafts will develop NIH, given enough time, not just vein grafts, but even prosthetic grafts that necessarily lack an antithrombotic endothelium. Vein grafts are deprived of their vasa vasorum during surgical harvest, leading to relative hypoxia and possibly reduced energy source; this adventitial injury induces the release of the inflammatory cytokines from SMC. Immediately after transplantation into the arterial environment, the venous wall is exposed to pulsatile flow with higher magnitudes and altered patterns of shear stress, injuring the vein graft endothelium; the responses to these environmental changes initiate vein graft adaptation, and may ultimately initiate NIH. Subsequent platelet aggregation, recruitment of the surrounding cells, and leukocyte migration with inflammatory responses contribute to the adaptive process. Vessel wall thrombus after endothelial injury also induces SMC proliferation. Later in the adaptive process, SMC migration and proliferation as well as extracellular matrix deposition directly contribute to remodeling but may eventually progress to NIH; the difference between wall thickening as a necessary adaptation process and the progressive pathological NIH that results in graft failure remains uncertain.

Since SMC play an essential role in vein graft adaptation as well as formation of NIH, the initial approach used to overcome vein graft failure in the seminal PREVENT trials was by regulation of SMC accumulation in the vein graft wall. Since the transcription factor E2F plays a pivotal role in the coordinated transactivation of cell cycle-regulatory genes that ultimately regulate SMC proliferation, double-stranded DNA with high affinity for E2F was introduced in vivo as a decoy to bind E2F and block the activation of genes mediating cell cycle progression and intimal hyperplasia; E2F decoy successfully inhibited vascular SMC proliferation in a rat carotid injury model. In a single center randomized controlled trial, intraoperative gene therapy using E2F decoy was tested in 41 patients undergoing infrainguinal arterial bypass with vein grafts;
in those patients receiving the E2F decoy, there were fewer graft occlusions, revisions or critical stenosis compared to the control group at 12 months.26,27)

Despite these promising initial results, the subsequent multicenter randomized trials, PREVENT-III and PREVENT-IV, resulted in disappointing failure. The PREVENT-III study, in patients with critical limb ischemia, showed no significant difference between the groups in the primary end points, time to nontechinical index graft reintervention or major amputation due to graft failure.30 The PREVENT-IV study, in patients having isolated coronary arterial bypass graft (CABG) surgery with at least two vein grafts, also failed; the primary end point, angiographic vein graft failure (≥75% vein graft stenosis) occurring 12 to 18 months after surgery, showed no significant difference between the groups.28) These results suggest that mechanisms of vein graft failure are more complex than just SMC proliferation,8,28,29) warranting further exploration.

Several preclinical studies have manipulated various intracellular signaling pathways that regulate vein graft NIH (Table 1). ERK is an important member of the MAPK pathway that plays an essential role during vein graft adaptation, and ERK inhibition decreased medial cell proliferation in canine vein grafts.30,31) Statins inhibit Rho that, with pulsatile stretch, accelerates eNOS expression; pravastatin treatment reduced NIH in rabbit vein grafts.32,33) Cyclic adenosine monophosphate response element binding protein (CREB), a nuclear transcription factor, regulates the expression of genes essential for cell proliferation,8,28,29) significantly repressed NIH in a mouse model.35) Mitogen Activated Protein Kinase Activated Protein Kinase II (MAPKAP2, MK2) is an intracellular kinase that stimulates CREB transcriptional activity; MMI-0100, MK2 inhibitor peptide, prevented murine vein graft thickening.36) Similarly, administration of Nogo-B also reduced NIH in a porcine model.37) Although these studies were successful in animal models, no strategy has translated into clinical use for patients; issues with translation include unknown and species-specificity of the mechanisms.38)

**Molecular Fingerprints of Arteries and Veins**

A novel strategy to combat vein graft failure focuses on membrane-bound signaling of venous endothelial cells in response to the arterial environment. Arteries and veins are anatomically distinguishable in the mature circulatory system. Arteries have a large diameter with a thick wall, exposed to high pressure and pulsatile flow of the highly oxygenated blood transported from the heart to peripheral tissues. Veins, on the other hand, work as a blood reservoir exposed to low pressure and relatively continuous flow towards the heart. The flexible thin wall of veins contributes to the adaptation to variable blood volume and their valves act to avoid blood reflux.

Histologically, all blood vessels have the same three-layer wall morphology: an internal intima, a media, and an external adventitia. These three layers are separated by the internal and external elastic laminae, at least in humans. Both the arterial and venous intima similarly consist of a single layer of endothelial cells lining the lumen of the vessel; however, the venous media has a significantly thinner layer of cellular and fibrous components, including circular smooth muscle cells that may contain collagen and some fibroblasts.

During embryogenesis, differentiation of undifferentiated cells into arterial or venous fate is regulated by the VEGF-delta-notch-Ephrin-Eph pathway (Fig. 2). Arterial differentiation of endothelial cell progenitors is initiated by activation of sonic hedgehog (Shh), a transcription factor that induces VEGF signaling; VEGF then stimulates its receptor VEGFR and co-receptor NP-1, which stimulate the Delta-Notch pathway in the endothelium,39,40) with delta-like ligand 4 (Dll4) ligand being one the first identified arterial markers.41) Dll-Notch then stimulates the arterial fate pathway by causing increased ephrin-B2 expression with simultaneous suppression of Eph-B4 expression; thus, Dll-Notch prevents acquisition of a venous fate.42) Interestingly, venous differentiation is not just a “default” pathway, but is also under active control; in cells destined to become veins, chicken ovalbumin upstream promoter transcription factor 2 (COUP-TFII) suppresses Notch and ephrin-B2,43) allowing expression of Eph-B4 and thus acquisition of venous identity.

Eph, named after its overexpression in a human

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**Table 1** Preclinical studies manipulating intracellular signaling to control neointimal hyperplasia in vein graft models

| Mechanism   | Treatment                  | Vein graft model (references) |
|-------------|----------------------------|------------------------------|
| ERK-1/2     | ERK-1/2 inhibitor          | Dog30,31)                    |
| PTEN        | PTEN adenovirus            | Dog34)                       |
| mTOR        | Rapamycin                  | Mouse85,86)                  |
| Rho         | Statin                     | Rabbit32,33)                 |
| CREB        | KCREB                      | Mouse35)                     |
| MAPKAP2     | MMI-0100                   | Mouse36)                     |
| Nogo-B      | Nogo-B adenovirus          | Pig37)                       |

PTEN: phosphatase and tensin homolog; CREB: cAMP responsive element-binding protein; KCREB: a CREB dominant protein; MAPKAP2: mitogen activated protein kinase activated protein kinase II; MMI-0100: MAPKAP2 inhibitor peptide; Nogo-B: neurite outgrowth inhibitor protein B
Erythropoietin-producing hepatocellular carcinoma cell line, was first discovered in a human cDNA library screen for sequences homologous to the tyrosine kinase domain of a viral oncogene. Eph receptors constitute the largest of the 14 families of tyrosine kinase receptors. Ephs, activated by their plasma-membrane-bound ligands ephrins, have many important functions during development and adulthood. Unlike the majority of receptor tyrosine kinases, bidirectional signaling can originate from both the ephrin ligand and the Eph receptor. Since both the ephrin ligand and the Eph receptor are tethered to the plasma membrane, the Eph-ephrin system seems to mediate cell-to-cell interactions directly, rather than via long-range communication. For example, the Eph-ephrin system contributes to vascular development, axon guidance, cell migration and tissue boundary formation. Ephrins can be divided into two subclasses, ephrin-A and ephrin-B, depending on their structural characteristics. Correspondingly, Eph receptors can be divided into Eph-A and Eph-B subclasses, based on their binding affinity to ephrins.

Members of the Eph-B subclass are transmembrane proteins. Remarkably, ephrin-B2 is specifically expressed by arteries while Eph-B4, one of its receptors, shows specific expression in veins. Ephrin-B2 acts both as a ligand and simultaneously as a receptor for Eph-B4. These reciprocal Eph-ephrin signaling pathways are active in endothelial cells at the arterial-venous capillary interface, and are critical for angiogenic remodeling and vessel development in the embryo. Endothelial cells expressing the arterial marker ephrin-B2 have limited ability to migrate ventrally, whereas those expressing the venous marker Eph-B4 preferentially move into the cardinal vein. Eph-B4 is also a critical regulator of early lymphatic vascular development, mutations in which can cause lymphatic dysplasia.

This specific expression pattern in arterial and venous endothelial cells persists into adulthood. Interestingly, as development proceeds, ephrin-B2 expression progressively extends from the arterial endothelium to surrounding SMC and pericytes, suggesting that ephrin-B2 may play an important role in adult neovascularization.

**Venous Identity is Lost in Vein Grafts**

Vein graft adaptation to the arterial environment involves wall thickening, fibrosis and subendothelial proliferation. Venous adaptation has been called, historically, "arterialization" of the vein. However, during rat vein graft adaptation, both Dll4 and Notch4 expression are downregulated, suggesting that "arterialization," e.g., acquisition of arterial markers, is not the correct terminology.

To determine how vessel identity is regulated during vein graft arterialization, we analyzed the pattern of ephrin-B2 and Eph-B4 expression in veins implanted into the arterial environment. Patent human saphenous vein grafts explanted from cardiac donors or limbs needing amputation showed reduced expression of Eph-B4 compared with native veins; Ephrin-B2, typically expressed at low levels in saphenous veins, was not induced in patent vein grafts. Similarly, in a rat vein graft model, which used jugular veins reversely interposed into carotid arteries, there was diminished Eph-B4, without increased ephrin-B2, compared with the native vein. A mouse vein graft model also showed similar findings, with reduced Eph-B4 expression and lack of ephrin-B2 induction.
of shear stress.\textsuperscript{62,63) These consistent results, in 3 in vivo models and an ex vivo model, demonstrate that vein graft adaptation results in loss of venous identity, but not in a gain of arterial identity (Fig. 3). Moreover, these studies imply that embryonic markers of vessel identity are plastic in adults and selective regulation of those markers may be capable of modifying the course of vein graft adaptation.

**Manipulation of Vessel Identity**

Based on these observations, we hypothesized that Eph-B4 regulates vein graft adaptation by inhibiting venous wall thickening; we also speculated that successful inhibition of wall thickening might reduce or delay neointimal hyperplasia and thus might be a reasonable strategy for clinical translation to improve the long-term outcome of vein grafts.

To test this hypothesis, we examined the effects of altered Eph-B4 signaling in a mouse model of vein graft adaptation.\textsuperscript{61} In this mouse model a thoracic IVC is transplanted into an infrarenal abdominal aorta of another mouse; vein graft wall thickness increased gradually up to 6 weeks after implantation with late positive remodeling by 12 weeks, recapitulating human vein graft adaptation. Mouse vein grafts also showed decreased Eph-B4 expression and unchanged ephrin-B2 expression.

**Downstream Effectors of Eph-B4 Signaling**

**Caveolin-1**

Caveolae are distinct flask-shaped invaginated structures that are located along the plasma membrane. They are recognized in the surface of many cell types including endothelial cells, and serve as signaling platforms. For example, Eph receptors enhance their signaling within caveolae. Caveolin-1 (Cav-1) is a major structural protein of caveolae in EC and is involved in mechanotransduction of dynamic shear stress changes by interacting with several signaling proteins, such as eNOS, ERK 1/2, and Eph receptors.\textsuperscript{64–66) To determine a mechanism of how Eph-B4 limits vein graft wall thickness, we examined Cav-1 and Eph-B4 interaction during vein graft adaptation. Eph-B4 stimulated phosphorylation of Cav-1 in EC, and phosphorylation of Cav-1 in vein grafts was correlated with Eph-B4 phosphorylation status; after activation of Eph-B4, colocalization and interaction of Eph-B4 and Cav-1 was detected. EC derived from Cav-1 knockout mice showed reduced Eph-B4-induced cell migration, and the thickened vein grafts derived from Cav-1 knockout mice were unresponsive to Eph-B4 stimulation. Vein grafts derived from mice with Cav-1 specifically reconstituted in their EC had greatly reduced thickness compared with those from Cav-1 knockout mice. These results suggest that Cav-1 mediates Eph-B4 signaling during venous adaptation, and that Eph-B4 regulation of vein graft thickening depends on endothelial Cav-1.

**Akt**

Phosphoinositide 3-kinases (PI3Ks) are protein and lipid kinases activated by different classes of membrane receptors; Akt is a major downstream effector of PI3K signaling.\textsuperscript{67} Akt activates by phosphorylation, and phospho-Akt regulates a network of downstream effectors, such as eNOS; the PI3K–Akt pathway has critical roles in regulating diverse cellular functions, such as cell survival, proliferation, and metabolism. PI3K is a potential Eph-receptor binding partner; in particular, PI3K binds to the EphA2 receptor.\textsuperscript{68} Migration and proliferation of human endothelial cells induced by activated Eph-B4 was inhibited in the presence of PI3K or Akt-inhibitors, suggesting that the PI3K–Akt pathway plays one of the central roles.
in Eph-B4 signaling in endothelial cells and vascular remodeling.\(^{49}\) In vitro, decreased Eph-B4 leads to activation of Akt; however, vein grafts had increased expression of both phosphorylated and total Akt compared with native veins but no change in Akt phosphorylation.\(^{70}\) These results suggest that the PI3K–Akt pathway also regulates vein graft remodeling but the differences between in vitro and in vivo results need additional study; juxtacrine and paracrine interactions between EC and SMC during vein graft adaptation complicate the interpretation of the data. In addition, Cav-1 and Akt have interactions that also require additional study.\(^{71,72}\)

### eNOS

Endothelial nitric oxide synthase (eNOS) is the nitric oxide synthase isoform by which nitric oxide (NO) is produced to regulate systemic blood pressure, vascular remodeling and angiogenesis.\(^{73}\) Impairment of eNOS-derived NO accelerates smooth muscle cell proliferation and migration that result in NIH.\(^{74,75}\) Eph-B4 regulates NO release in endothelial cells; after ephrin-B2/Fc stimulation, eNOS phosphorylation and NO production are increased in human endothelial cells,\(^{76}\) while mouse endothelial cells derived from heterozygous Eph-B4 mice have less NO release compared with WT EC.\(^{70}\)

Since NO is a known regulator of vein graft wall thickness,\(^{77}\) we used our mouse vein graft model to determine if eNOS is a downstream mediator of Eph-B4 signaling during vein graft adaptation.\(^{78}\) Activation of Eph-B4 with ephrin-B2/Fc stimulated eNOS phosphorylation and cell migration in vitro, which was abolished with eNOS inhibition. In vivo, the decreased Eph-B4 expression that occurs during vein graft adaptation correlates with increased eNOS activity; in eNOS knockout vein grafts, venous remodeling is reduced and Eph-B4 activity is enhanced. These data suggest that Eph-B4 regulates endothelial cell functions mediated by eNOS phosphorylation and that eNOS mediates venous remodeling during vein graft adaptation.

However, the discordance of eNOS regulation by Eph-B4 between the in vitro and in vivo studies remains unresolved. Diverse mediators such as Src\(^{79}\) and Rho kinase\(^{80}\) may play a role in regulating the Eph-B4–eNOS pathway in vivo, inducing a more complex interaction or additional responses in different cell types. In addition, Akt directly mediates eNOS activation, leading to increased NO production in EC.\(^{81}\) Increased eNOS-Cav1 interaction negatively regulates eNOS phosphorylation.\(^{79}\) Co-immunoprecipitation studies show that nearly all the eNOS in endothelial cells is associated with Cav-1.\(^{82}\) The Cav-1 scaffolding domain serves as an endogenous negative regulator of eNOS function.\(^{83}\)

Other signaling associated with Eph-B4–eNOS pathway were manipulated to regulate NIH (Table 1, Fig. 4). PTEN, a downstream inhibitor of PI3K, prevented Akt phosphorylation and limits NIH by decreasing SMC proliferation.\(^{84}\) Rapamycin, an mTOR inhibitor, regulated NIH via PI3K–Akt signal suppression.\(^{85,86}\) Interestingly, Eph-B4 regulates the Ras/ERK pathway in EC\(^{87}\) (Fig. 4).

### Manipulation of Eph-B4 in Human Saphenous Veins

Autologous saphenous vein remains the most commonly used and durable conduit for arterial bypass. To determine whether Eph-B4 is functional in human veins, as it...
is in adult murine veins, we stimulated discarded human saphenous veins with ephrin-B2/Fc. Eph-B4 activation was associated with reduced neointimal thickening in a human saphenous vein ring assay. Stimulation of Eph-B4 in human endothelial cells induced phosphorylation of Eph-B4 and Cav-1, and release of NO. Moreover, adventitial delivery of ephrin-B2/Fc followed by 24 hours of arterial shear stress increased endothelial Eph-B4 phosphorylation. These results show that human saphenous veins have Eph-B4 and that it is functional; this data also supports the concept that regulation of Eph-B4 may be a strategy of translational potential for human patients needing vein grafts.

Conclusions

Eph-B4, the venous determinant expressed during embryonic development, remains expressed and functional in adults. Although the normal functions of Eph-B4 in adult veins are not yet clear, loss of Eph-B4 expression during vein graft adaptation suggests that Eph-B4 regulates venous wall thickness. Strategies to alter Eph-B4 activity, or its downstream effectors such as caveolin-1, Akt, and eNOS, may be translatable as a strategy to inhibit NIH that is currently the most important mechanism of vein graft failure. However, better understanding of the factors that distinguish favorable and unfavorable venous remodeling is needed before designing the next clinical trial to prevent vein graft failure.

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Disclosure Statement

All authors have no conflict of interest.

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References

1) Kunlin J. Le traitement de l’arterite obliterante par la greffe veineuse. Arch Mal Coeur. 1949; 42: 371-2.
2) Kunlin J. Le traitement de l’ischémie arteriétique par la greffe veineuse longue. Rev Chir 1951; 70: 206-35.
3) King P, Royle JP. Autogenous vein grafting in atheromatous rabbits. Cardiovasc Res 1972; 6: 627-33.
4) Wyatt AP, Rothnie NG, Taylor GW. The vascularization of vein-grafts. Br J Surg 1964; 51: 378-81.
5) Menzoian JO, Koshar AL, Rodrigues N. Alexis Carrel, Rene Leriche, Jean Kunlin, and the history of bypass surgery. J Vasc Surg 2011; 54: 571-4.
6) Pereira CE, Albers M, Romiti M, et al. Meta-analysis of femoropopliteal bypass grafts for lower extremity arterial insufficiency. J Vasc Surg 2006; 44: 510-7.e3.
7) Johnson WC, Lee KK. A comparative evaluation of polytetrafluoroethylene, umbilical vein, and saphenous vein bypass grafts for femoral-popliteal above-knee revascularization: a prospective randomized Department of Veterans Affairs cooperative study. J Vasc Surg 2000; 32: 268-77.
8) Conte MS, Bandyk DF, Clowes AW, et al.; PREVENT III Investigators. Results of PREVENT III: a multicenter, randomized trial of edifoligide for the prevention of vein graft failure in lower extremity bypass surgery. J Vasc Surg 2006; 43: 742-51.e1.
9) Owens CD. Adaptive changes in autogenous vein grafts for arterial reconstruction: clinical implications. J Vasc Surg 2010; 51: 736-46.
10) Owens CD, Gasper WJ, Rahman AS, et al. Vein graft failure. J Vasc Surg 2015; 61: 203-16.
11) Kikuchi S, Kenagy RD, Gao L, et al. Surgical marking pen dye inhibits saphenous vein cell proliferation and migration in saphenous vein graft tissue. J Vasc Surg 2016; 63: 1044-50.
12) Eagle S, Brophy CM, Komalavilas P, et al. Surgical skin markers impair human saphenous vein graft smooth muscle and endothelial function. Am Surg 2011; 77: 922-8.
13) Schanzer A, Hevelone N, Owens CD, et al. Technical factors affecting autogenous vein graft failure: observations from a large multicenter trial. J Vasc Surg 2007; 46: 1180-90; discussion, 90.
14) Owens CD, Wake N, Jacot JG, et al. Early biomechanical changes in lower extremity vein grafts—distinct temporal phases of remodeling and wall stiffness. J Vasc Surg 2006; 44: 740-6.
15) Langille BL, O’Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. Science 1986; 231: 405-7.
16) Imparato AM, Bracco A, Kim GE, et al. Intimal and neointimal fibrous proliferation causing failure of arterial reconstructions. Surgery 1972; 72: 1007-17.
17) Clowes AW, Kohler T. Graft endothelialization: the role of angiogenic mechanisms. J Vasc Surg 1991; 13: 734-6.
18) Zhang L, Hagen P-O, Kisslo J, et al. Neointimal hyperplasia rapidly reaches steady state in a novel murine vein graft model. J Vasc Surg 2002; 36: 824-32.
19) Owens CD, Rybicki FJ, Wake N, et al. Early remodeling of
lower extremity vein grafts: inflammation influences biomechanical adaptation. J Vasc Surg 2008; 47: 1235-42.

20) Kudo FA, Muto A, Maloney SP, et al. Venous identity is lost but arterial identity is not gained during vein graft adaptation. Arterioscler Thromb Vasc Biol 2007; 27: 1562-71.

21) LoGerfo FW, Quist WC, Nowak MD, et al. Downstream anastomotic hyperperfusion. A mechanism of failure in Dacron arterial grafts. Ann Surg 1983; 197: 479-83.

22) de Graaf R, Tintu A, Stassen F, et al. N-acetylcysteine prevents neointima formation in experimental venous bypass grafts. Br J Surg 2009; 96: 941-50.

23) Muto A, Model I, Ziegler K, et al. Mechanisms of vein graft adaptation to the arterial circulation: insights into the neointimal algorithm and management strategies. Circulation Journal: Official Journal of the Japanese Circulation Society 2010; 74: 1501-12.

24) Hu H, Patel S, Hansich J, et al. Future research directions to improve fistula maturation and reduce access failure. Semin Vasc Surg. Epub Aug 25, 2016. DOI: http://dx.doi.org/10.1053/j.semvascsurg.2016.08.005

25) Morishita R, Gibbons GH, Horiuchi M, et al. A gene therapy strategy using a transcription factor decoy of the E2F binding site inhibits smooth muscle proliferation in vivo. Proc Natl Acad Sci USA 1995; 92: 5855-9.

26) Mann MJ, Whitemore AD, Donaldson MC, et al. Ex-vivo gene therapy of human vascular bypass grafts with E2F decoy: the PREVENT single-centre, randomised, controlled trial. Lancet (London, England) 1999; 354: 1493-8.

27) Conte MS, Mann MJ, Simosa HF, et al. Genetic interventions for vein bypass graft disease: a review. J Vasc Surg 2002; 36: 1040-52.

28) Lopes RD, Williams JB, Mehta RH, et al. Edifoligide and long-term outcomes after coronary artery bypass grafting: PRoject of Ex-vivo Vein graft ENgineering via Transfection IV (PREVENT IV) 5-year results. Am Heart J 2012; 164: 379-86.e1.

29) Alexander JH, Haflay G, Harrington RA, et al.; PREVENT IV Investigators. Efficacy and safety of edifoligide, an E2F transcription factor decoy, for prevention of vein graft failure following coronary artery bypass graft surgery: PREVENT IV: a randomized controlled trial. JAMA 2005; 294: 2446-54.

30) Pintucci G, Saunders PC, Gulkarov I, et al. Anti-proliferative and anti-inflammatory effects of topical MAPK inhibition in arterialized vein grafts. FASEB J 2006; 20: 398-400.

31) Sharony R, Pintucci G, Saunders PC, et al. Matrix metalloproteinase expression in vein grafts: role of inflammatory mediators and extracellular signal-regulated kinases-1 and -2. Am J Physiol Heart Circ Physiol 2005; 290: H1651-9.

32) Yamanouchi D, Banno H, Nakayama M, et al. Hydrophilic statin suppresses vein graft intimal hyperplasia via endothelial cell-tropic Rho-kinase inhibition. J Vasc Surg 2005; 42: 737-44.

33) Fujita H, Banno H, Yamanouchi D, et al. Pitavastatin inhibits intimal hyperplasia in rabbit vein graft. J Surg Res 2008; 148: 238-43.

34) Johannessen M, Delhlandi MP, Moens U. What turns CREB on? Cell Signal 2004; 16: 1211-27.

35) Nakanishi K, Saito Y, Azuma N, et al. Cyclic adenosine monophosphate response-element binding protein activation by mitogen-activated protein kinase-activated protein kinase 3 and four-and-a-half LIM domains 5 plays a key role for vein graft intimal hyperplasia. J Vasc Surg 2013; 57: 182-93, 193.e1-10.

36) Muto A, Panitch A, Kim N, et al. Inhibition of mitogen activated protein kinase activated protein kinase II with MM1-0100 reduces intimal hyperplasia ex vivo and in vivo. Vascul Pharmacol 2012; 56: 47-55.

37) Kritz AB, Yu J, Wright PL, et al. In vivo modulation of Nogo-B attenuates neointima formation. Mol Ther 2008; 16: 798-804.

38) Zheng H, Xue S, Lian F, et al. A novel promising therapy for vein graft restenosis: overexpressed Nogo-B induces vascular smooth muscle cell apoptosis by activation of the JNK/p38 MAPK signaling pathway. Med Hypotheses 2011; 77: 278-81.

39) Lawson ND, Scheer N, Pham VN, et al. Notch signaling is required for arterial-venous differentiation during embryonic vascular development. Development 2001; 128: 3675-83.

40) Lawson ND, Vogel AM, Weinstein BM. sonic hedgehog and vascular endothelial growth factor act upstream of the Notch pathway during arterial endothelial differentiation. Dev Cell 2002; 3: 127-36.

41) Shutter JR, Scully S, Fan W, et al. Dll4, a novel Notch ligand expressed in arterial endothelium. Genes Dev 2000; 14: 1313-8.

42) Siekmann AF, Lawson ND. Notch signalling and the regulation of angiogenesis. Cell Adhes Migr 2007; 1: 104-5.

43) You LR, Lin FJ, Lee CT, et al. Suppression of Notch signaling by the COUP-TFII transcription factor regulates vein identity. Nature 2005; 435: 98-104.

44) Hirai H, Maru Y, Hagiwara K, et al. A novel putative tyrosine kinase receptor encoded by the eph gene. Science 1987; 238: 1717-20.

45) Kullander K, Klein R. Mechanisms and functions of eph and ephrin signalling. Nat Rev Mol Cell Biol 2002; 3: 475-86.

46) Davis S, Gale NW, Aldrich TH, et al. Ligands for EPH-related receptor tyrosine kinases that require membrane attachment or clustering for activity. Science 1994; 266: 816-9.

47) Orioli D, Klein R. The Eph receptor family: axonal guidance and vascular morphogenesis. Development 2001; 128: 2941-5.

48) Adams RH, Wilkinson GA, Weiss C, et al. Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. Genes Dev 1999; 13: 295-306.

49) Gale NW, Holland SJ, Valenzuela DM, et al. Eph receptors and ligands comprise two major specificity subclasses and are reciprocally compartmentalized during embryogenesis. Neuron 1996; 17: 9-19.

50) Torres-Vázquez J, Kamei M, Weinstein BM. Molecular distinction between arteries and veins. Cell Tissue Res 2003; 314: 43-59.

51) Wang HU, Chen ZF, Anderson DJ, et al. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. Cell 1998; 93: 741-53.

52) Kim I, Ryu YS, Kwak HJ, et al. EphB ligand, ephrinB2, suppresses the VEGF- and angiopepoin-1-induced Ras/mitogen-activated protein kinase pathway in venous endothelial cells.
53) Herbert SP, Huisken J, Kim TN, et al. Arterial-venous segregation by selective cell sprouting: an alternative mode of blood vessel formation. Science 2009; 326: 294-8.

54) Pitulescu ME, Adams RH. Eph/ephrin molecules—a hub for signaling and endocytosis. Genes Dev 2010; 24: 2480-92.

55) Martin-Almedina S, Martinez-Corraal I, Holdhus R, et al. EphB4 kinase-inactivating mutations cause autosomal dominant lymphatic-related hydrops fetalis. J Clin Invest 2016; 126: 3080-8.

56) Hashimoto T, Tsuneki M, Foster TR, et al. Membrane-mediated regulation of vascular identity. Birth Defects Res C Embryo Today 2016; 108: 65-84.

57) Shin D, Garcia-Cardena G, Hayashi S-I, et al. Expression of ephrinB2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. Dev Biol 2001; 230: 139-50.

58) Gale NW, Baluk P, Pan L, et al. Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. Dev Biol 2001; 230: 151-60.

59) Fancher TT, Muto A, Fitzgerald TN, et al. Control of blood vessel identity: from embryo to adult. Ann Vasc Dis 2008; 1: 28-34.

60) Bush HL Jr, Jakubowski JA, Curl GR, et al. The natural history of endothelial structure and function in arterialized vein grafts. J Vasc Surg 1986; 3: 204-15.

61) Muto A, Yi T, Harrison KD, et al. Eph-B4 prevents venous arterialization via the phosphatidylinositol 3-kinase pathway. J Biol Chem 1994; 269: 39930-5.

62) Model LS, Hall MR, Wong DJ, et al. Arterial shear stress reduces eph-b4 expression in adult human veins. Yale J Biol Med 2014; 87: 359-71.

63) Berard X, Deglise S, Alonso F, et al. Role of hemodynamic forces in the ex vivo arterialization of human saphenous veins. J Vasc Surg 2013; 57: 1371-82.

64) Yu J, Bergaya S, Murata T, et al. Direct evidence for the role of caveolin-1 and caveoleae in mechanotransduction and remodeling of blood vessels. J Clin Invest 2006; 116: 1284-91.

65) Gratton JP, Fontana J, O’Connor DS, et al. Reconstitution of an endothelial nitric-oxide synthase (eNOS), hsp90, and caveolin-1 complex in vitro. Evidence that hsp90 facilitates calmodulin stimulated displacement of eNOS from caveolin-1. J Biol Chem 2000; 275: 22268-72.

66) Rivera M, Muto A, Feigel A, et al. Venous and arterial identity: a role for caveoleae? Vascular 2009; 17 Suppl. 1: S10-4.

67) Graupera M, Potente M. Regulation of angiogenesis by PI3K signaling networks. Exp Cell Res 2013; 319: 1348-55.

68) Pandey A, Lazar DF, Saltiel AR, et al. Activation of the Eck receptor protein tyrosine kinase stimulates phosphatidylinositol 3-kinase activity. J Biol Chem 1994; 269: 30154-7.

69) Steinle JJ, Meininger CJ, Forough R, et al. Eph B4 receptor signaling mediates endothelial cell migration and proliferation via the phosphatidylinositol 3-kinase pathway. J Biol Chem 2002; 277: 43830-5.

70) Jadlowiec CC, Feigel A, Yang C, et al. Reduced adult endothelial cell EphB4 function promotes venous remodeling. Am J Physiol Cell Physiol 2013; 304: C627-35.

71) Park JH, Han HJ. Caveolin-1 plays important role in EGF-induced migration and proliferation of mouse embryonic stem cells: involvement of PI3K/Akt and ERK. Am J Physiol Cell Physiol 2009; 297: C935-44.

72) Zhang B, Peng F, Wu D, et al. Caveolin-1 phosphorylation is required for stretch-induced EGFR and Akt activation in mesangial cells. Cell Signal 2009; 19: 1690-700.

73) Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J 2012; 33: 829-37, 837a-837d.

74) Sugimoto M, Yamanouchi D, Komori K. Therapeutic approach against intimal hyperplasia of vein grafts through endothelial nitric oxide synthase/nitric oxide (eNOS/NO) and the Rho/Rho-kinase pathway. Surg Today 2009; 39: 459-65.

75) Rudic RD, Shesely EG, Maeda N, et al. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. J Clin Invest 1998; 101: 731-6.

76) Wong DJ, Lu DY, Protack CD, et al. Ephrin type-B receptor 4 activation reduces neointimal hyperplasia in human saphenous vein in vitro. J Vasc Surg 2016; 63: 795-804.

77) Kibble MR, Tzeng E, Gleixner SL, et al. Adenovirus-mediated gene transfer of human inducible nitric oxide synthase in porcine vein grafts inhibits intimal hyperplasia. J Vasc Surg 2001; 34: 156-65.

78) Wang M, Collins MJ, Foster TR, et al. Eph-B4 mediates vein graft adaptation by regulation of endothelial nitric oxide synthase. J Vasc Surg 2017; 65: 179-89.

79) Chen Z, Bakhshi FR, Shahjahan AN, et al. Nitric oxide-dependent Src activation and resultant caveolin-1 phosphorylation promote eNOS/caveolin-1 binding and eNOS inhibition. Mol Biol Cell 2012; 23: 1388-98.

80) Shamah SM, Lin MZ, Goldberg JL, et al. EphA receptors regulate growth cone dynamics through the novel guanine nucleotide exchange factor ephexin. Cell 2001; 105: 233-44.

81) Fulton D, Gratton JP, McCabe TJ, et al. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature 1999; 399: 597-601.

82) Feron O, Belhassen L, Kobzik L, et al. Endothelial nitric oxide synthase targeting to caveoleae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. J Biol Chem 1996; 271: 22810-4.

83) Bernatchez P, Sharma A, Bauer PM, et al. A noninhibitory mutant of the caveolin-1 scaffolding domain enhances eNOS-derived NO synthesis and vasodilation in mice. J Clin Invest 2011; 123: 3747-55.

84) Hata JA, Petrofski JA, Schroder JN, et al. Modulation of phosphatidylinositol 3-kinase signaling reduces intimal hyperplasia in aortocoronary saphenous vein grafts. J Thorac Cardiovasc Surg 2005; 129: 1405-13.

85) Schachner T, Oberhuber A, Zou Y, et al. Rapamycin treatment is associated with an increased apoptosis rate in experimental vein grafts. Eur J Cardiothorac Surg 2003; 27: 302-6.

86) Schachner T, Zou Y, Oberhuber A, et al. Local application of rapamycin inhibits neointimal hyperplasia in experimental vein grafts. Ann Thorac Surg 2004; 77: 1580-5.

87) Xiao Z, Carrasco R, Kinne K, et al. EphB4 promotes or suppresses Ras/MEK/ERK pathway in a context-dependent manner: implications for EphB4 as a cancer target. Cancer Biol Ther 2012; 13: 630-7.