Narrowing down graft stenosis

By Benjamin Boettner, Associate Editor

One of the dominant problems after coronary artery bypass graft surgery is hyperplastic growth inside the transplanted blood vessel that can lead to stenosis. Now, a team at the **NIH**'s **National Heart, Lung, and Blood Institute** has found that this hyperplasia is rooted in a change in the phenotype of endothelial cells lining the transplanted veins and has shown that blocking transforming growth factor-β activity in mice can significantly slow the cellular changes.[1]

Next, the team will try to replicate the findings using transplant models in pigs and will investigate the transforming growth factor-β (TGFB; TGFβ) pathway for other points of intervention that could represent therapeutic targets.

Cellular changes are a fundamental part of the vascular remodeling process after coronary artery bypass graft (CABG) surgery. The surgery involves grafting a blood vessel—often a vein—to an atherosclerotic coronary artery, thus creating a new route for blood to flow that bypasses the blockage.

However, about 40% of vein grafts fail within two years of surgery, frequently because of adverse vascular remodeling during which cell masses—termed neointima—build up in the walls of the grafted veins. The neointima are formed largely by migrating and proliferating cells that resemble vascular smooth muscle cells (VSMCs).

There are no treatments for prevention of long-term graft failure, and there has been little progress in designing therapies to treat or prevent neointimal hyperplasia because neither the mechanisms controlling neointima formation nor the origins of the cells involved have been well understood.

Now, a team led by Manfred Boehm at the National Heart, Lung, and Blood Institute has used a fluorescence-based vein transplantation model in mice to track changes in endothelial cells in the grafted vessels and has found that endothelial cells take on properties of mesenchymal cells. The endothelial-to-mesenchymal transition yields cells with VSMC-like features and appears to be controlled by TGFβ signaling, which could provide a handle for developing targeted therapies.

Boehm is a senior investigator at the National Heart, Lung, and Blood Institute.

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> ***—Angela Bradshaw, University of Glasgow Institute of Cardiovascular and Medical Sciences***

Neointima-te details

To find out whether neointima derive from the graft itself or are formed by VSMCs from distant sites in the recipient mice, Boehm's team removed jugular veins containing fluorescently labeled endothelia from transgenic donor mice and grafted them into the femoral artery of nonfluorescent recipient mice.

Neointima formed following the surgery and contained a large proportion of cells derived from the grafted fluorescent endothelial cells. The number of endothelial lineage cells in the neointima increased throughout the 35-day monitoring period, whereas levels of other cells in the neointima reached a plateau by day 14 post-surgery.

The team then characterized the fluorescent cells from the neointima using markers specific for endothelia, including platelet/endothelial cell adhesion molecule (Pecam1; Cd31) and VE-cadherin (Cd144; cadherin-5), and markers specific for immature VSMCs, including actin α2 smooth aorta muscle (Acta2; α-sma) and transgelin (Tagln; Sm22).

In the first few days after surgery, the neointima cells expressed mainly endothelial markers. But they contained predominantly immature VSMC markers by day 35.

The researchers found similar results in human tissue samples from early phase failed vein grafts, which expressed the immature VSMC markers α-SMA and SM22 in addition to endothelial markers.

They concluded that the grafted endothelial cells had undergone a progressive shift in phenotype—which represented an endothelial-to-mesenchymal transition—and that 85% of the neointimal cells had a mesenchymal phenotype by the end of the observation period.

Next, the team looked for the molecular pathway driving the phenotypic change.

They focused on TGFβ-mediated signaling because the cytokine had previously been implicated in vascular remodeling in developmental and fibrotic processes and is thought to regulate thickening of the endothelial lining after vascular injury.[2,3]

The team found that TGFβ-regulated smad family member 2 (Madh2; Smad2) and Smad3 (Madh3) activated the snail family zinc finger 2 (Snai2; Slug; Snail2) transcription factor to drive the endothelial-to-mesenchymal transition. In addition, shRNA against *Smad2* or *Smad3* in mouse veins prior to performing the graft decreased the formation of neointima by day 35 post-surgery compared with control shRNA.

Finally, the team tested whether blocking the pathway pharmacologically could affect the vascular remodeling. TGFβ-neutralizing antibodies produced smaller neointimal areas and fewer endothelial-derived cells in graft-transplanted mice than control antibodies. The team concluded that inhibiting TGFβ could decrease neointima formation after transplanting vein grafts.

The findings were published in *Science Translational Medicine*.

Of pigs and men

Angela Bradshaw, a research fellow at the **University of Glasgow Institute**

of Cardiovascular and Medical Sciences, told *SciBX*, "The study has undoubtedly made an important contribution to our understanding of how TGFβ promotes vein graft neointima formation."

However, she said that further studies on the mechanism are needed because other TGFβ-induced pathways such as the Smad1 (Madh1), Smad5 (Madh5) and Smad9 (Madh9) pathway, which is triggered in endothelial cells by the activin receptor-like kinase 1 (Acvrl1; Alk1; Hht2) receptor, may have opposite effects to Smad2 and Smad3.

"As TGFβ itself is such a multifunctional protein, systemic treatment would have to be kept to a minimum. It would be interesting to test whether pretreatment of vein grafts alone before surgery is sufficient to exert therapeutic or beneficial effects."
—Peter ten Dijke, Leiden University

Boehm said that his team plans to continue studying the molecular pathway *in vitro* to tease out possible synergies or antagonisms with the aim of identifying an optimal target.

Peter ten Dijke suggested that researchers test whether pretreatment of the grafts can have therapeutic effects and thereby circumvent the wide range of effects of TGFβ inhibitors when applied systemically. "As TGFβ itself is such a multifunctional protein, systemic treatment would have to be kept to a minimum. It would be interesting to test whether pretreatment of vein grafts alone before surgery is sufficient to exert therapeutic or beneficial effects," he said.

He added that existing drugs that interfere with TGFβ signaling should be tested, such as Cozaar losartan—a systemic inhibitor of angiotensin-induced activation of TGFβ signaling.

ten Dijke is a professor at **Leiden University** and group leader at the **Leiden University Medical Center**, where he studies TGFβ signaling in diverse disease processes.

Cozaar reduced aortic dilation rate in the recent COMPARE (COzaar in Marfan PAtients Reduces aortic Enlargement) trial on patients with Marfan syndrome—a genetic connective tissue disease that affects the heart valves and aorta and is driven by ectopic TGFβ signaling.

Cozaar is an angiotensin II type 1 receptor (AGTR1) antagonist marketed by **Merck & Co. Inc.** to treat hypertension, heart failure and stroke.

ten Dijke and Anita Thomas both told *SciBX* that further studies in additional species would be needed to translate the findings for clinical applications.

According to Thomas, although the mouse model of vein grafting allows lineage tracing, its main disadvantage is that at one month after surgery, the graft is quite different from the vein that was grafted into position.

"Very few of the original cells remain in the graft, and most of the extracellular matrix proteins will have been altered during the cellular repopulation of the graft and vessel wall remodeling," she said. "The use of a large-animal model of vein grafting, such as the carotid artery end-to-end or end-to-side model in the pig, would effectively eliminate this problem since grafts in pig models are much thicker, containing more cell layers and are more stable over time."

Thomas is a research associate at the **University of Bristol School of Clinical Sciences**, where she investigates therapeutic developments in cardiovascular disease.

ten Dijke added that studying endothelial-to-mesenchymal transition in other human pathologies such as cerebellar cavernous malformations, Kaposi sarcoma herpesvirus–induced endothelial transformation or tissue fibrosis could also benefit the understanding of adverse vascular remodeling in CABGs.

Boehm told *SciBX* that his team is suggesting further *in vivo* studies using larger animals including pigs. In addition, he said that the findings can be bolstered by examining changes caused by arteriovenous shunts in patients with kidney disease and patients with peripheral arteriovenous bypasses, who tend to have even worse outcomes than CABG recipients.

The findings have not been patented.

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