Emerging Role of IGF-1R in Stretch-Induced Neointimal Hyperplasia in Venous Grafts

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Autologous saphenous veins are commonly used to circumvent occluded coronary arteries during coronary artery bypass grafting (CABG) procedures. Although these vessels provide an effective treatment for myocardial ischemic disease, they are susceptible to accelerated atherosclerosis and fail in 50% of cases within 10 years of surgery. Venous graft disease occurs via 3 temporally distinct processes including thrombosis, neointimal hyperplasia, and atherosclerosis with each process contributing significantly to the onset of the next. During neointimal hyperplasia, vascular smooth muscle cells (VSMCs) and extracellular matrix accumulate in the intimal region causing intimal thickening and narrowing of the vessel lumen. Even though the disease process has been described, the detailed mechanisms behind the cellular events are unclear. In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Cheng and Du illustrate a novel underlying mechanism involving an insulin-like growth factor (IGF-1) and IGF-1 receptor (IGF-1R) pathway by which cyclic stretch of VSMC plays a critical role in intimal thickening of venous grafts.

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Blood flow generates a wide range of hemodynamic forces including frictional wall shear stress, circumferential stress, and compressive stress. Arteries are exposed to cyclic circumferential stretch attributable to pulsatile blood flow. Furthermore, they experience higher pressures than veins. Whereas the endothelium is constantly subjected to fluid shear stress, the underlying layers of smooth muscle cells are exposed mainly to circumferential stretch. It has been well established that altered mechanical forces cause dysregulated cellular phenotypes and contribute to pathology in disease conditions such as hypertension, atherosclerosis, and venous graft failure. When a vein is grafted to replace a diseased artery, it becomes exposed to substantially higher pressure and flow. This contributes to increased wall shear stress and pulsatile circumferential stretch, promoting hyperplasia. However, the detailed molecular mechanisms responsible for this VSMC proliferation and neointimal hyperplasia by mechanical stretch are not well understood.

VSMC proliferation is regulated by numerous growth factors including platelet derived growth factor (PDGF), basic and acidic fibroblast growth factor (FGF), IGF-1, epidermal growth factor (EGF), endothelin-1, and angiotensin II. Of these, IGF-1 seems to play a pivotal role, because neutralizing anti-IGF-1 antibodies can prevent VSMC proliferation induced by other growth factors including angiotensin II, thrombin, bFGF, and serum. These results suggest that many growth factors promoting VSMC proliferation do so by inducing production of IGF-1, which acts as an autocrine factor. It is also well known that the VSMC growth factors mentioned above are expressed in venous grafts. Based on these findings, Cheng and Du hypothesized that IGF-1 would play a key role in stretch-induced venous SMC proliferation, leading to neointimal thickening in venous grafts.

IGF-1 binds to IGF-1R, initiating various intracellular signaling cascades including activation of IRS family members and Shc. The diverse physiological effects of IGF-1R activation include differentiation, proliferation, inhibition of apoptosis, reactive oxygen species (ROS) production, and cellular transformation. However, in some cases, growth factor receptors can be activated in a ligand-independent manner. For example, PDGF receptor α and EGF receptor can be activated by stretch in a ligand-independent manner in VSMCs. In addition, shear stress can activate VEGF receptor-2 without requiring VEGF in endothelial cells. Can the IGF-1R also be activated in a similar manner? In this issue, Cheng and Du show compelling evidence that IGF-1R plays a critical role in stretch-induced vascular hypertrophy in a ligand-independent and -dependent manner.

In their current article, Cheng and Du carried out a series of elegant and extensive studies to demonstrate the role of IGF-1 and IGF-1R in SMC proliferation and neointimal thickening of venous grafts. First, they successfully generated transgenic mice deficient in IGF-1R only in adults by using a tamoxifen-inducible Cre-LoxP system (ER-Cre and floxed IGF-1R). This inducible strategy was necessary to circumvent severe developmental problems and perinatal lethality caused by its knockout at the embryonic stage. When adult mice were injected with tamoxifen, IGF-1R was efficiently deleted in the mice. Using vena cavae from these mice, they then cultured venous SMCs for in vitro cyclic stretch studies. For venous graft studies, vena cavae from the IGF-1R null mice were grafted to the right common carotid artery, and studied after 4 weeks with immunohistochemical methods. As illustrated in the Figure, mechanical stretch of venous SMCs increased IGF-1 and IGF-1R expression in a time-dependent manner both at the mRNA and protein level. IGF-1 mRNA increase was evident at 4 hours and peaked at 8 hours,

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after a 2-hour stretch. IGF-1R mRNA level peaked at an earlier time point (2 hours) than IGF-1. They then demonstrated that IGF-1R is tyrosine phosphorylated (indicated as pTyr in the Figure) in a bi-phasic manner by stretch: IGF-1R tyrosine phosphorylation was increased early (6 minutes to 2 hours), decreased back to basal by 12 hours, and increased again by 24 hours. The next series of studies demonstrated, using both pharmacological and molecular approaches, that IGF1-independent and -dependent mechanisms were responsible for the acute and late phosphorylation of IGF-1R, respectively. Studies further showed that stretch activated Src, which in turn rapidly activated IGF-1R, IRS-1, PI3k, and Akt, all in an IGF1-independent manner. In contrast, IGF-1 directly produced by stretch seems to be responsible for the late phase activation of IGF-1R and IRS-1. Their data imply that both the early and late phase responses lead to SMC proliferation. Consistent with the in vitro findings, animal studies showed that neointimal hyperplasia occurring in the venous graft was significantly blunted in the IGF-1R null mice.

Previously, Standley et al showed that cyclic stretch of rat aortic SMCs increased cell proliferation and IGF-1 expression, and a neutralizing anti–IGF-1 antibodies prevented this stretch-induced proliferation. In vivo studies have also suggested an important role for IGF-1. However, these studies did not explore a role for IGF-1R in the pathway. The current article by Cheng and Du brings to the forefront a vital role for IGF-1R in stretch-induced VSMC proliferation. As with any significant finding, this work points to several new research directions and some unanswered questions: What are the mechanosensors that transduce mechanical stretch of SMC into both early and late signaling pathways in a Src-dependent and -independent manner, respectively? What is the hierarchical relationship between the early response of Src/IGF-1R/IRS-1 pathway and the increased IGF-1R expression? In addition, it remains to be demonstrated whether the neointimal thickening observed in the venous graft model leads to atheroma formation or occlusion.
In summary, Cheng and Du present a novel mechanism by which IGF-1 and IGF-1R play key roles in stretch-induced SMC proliferation and neointimal thickening. This insight may provide a new therapeutic avenue by targeting IGF or IGF-1R to prevent venous graft failure and vascular restenosis.

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