ATF-4 and Vascular Injury
Integration of Growth Factor Signaling and the Cellular Stress Response

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Smooth muscle proliferation and neointimal formation are characteristic features of vascular lesions that develop after vascular injury and contribute to the development of occlusive vascular lesions after percutaneous coronary interventions and in transplant vasculopathy. The traditional model for the development of these vascular lesions has postulated that a complex interplay among locally released growth factors and cytokines, circulating platelets and inflammatory cells, local smooth muscle and endothelial cells, and perhaps circulating precursor cells involving multiple cellular processes such as adhesion, proliferation, migration, and apoptosis is orchestrated at many levels, resulting in vascular stenosis. The temporal and spatial complexity of the overall process and the diverse contributions of various components have provided many opportunities for study, but identifying and linking the various critical steps in the development of occlusive vascular lesions has remained challenging. Although numerous studies examining roles of vascular signaling pathways and vascular transcription factors have been published, a clear picture of how signaling and transcription are intertwined remains elusive. In this issue of Circulation Research, Malabanan et al present a comprehensive series of experiments linking the transcription factor activating transcription factor (ATF)-4 to intimal thickening after injury and identify both upstream and downstream associated growth factors and cytokines, circulating platelets and inflammatory cells.

The authors initially identified ATF-4 as a potential regulator of neointimal formation through a microarray screen for genes induced in smooth muscle cells (SMCs) by fibroblast growth factor (FGF)-2. FGF-2 has been identified previously as a major smooth muscle mitogen and has been implicated in the pathogenesis of atherosclerosis and restenosis after angioplasty. FGF-2 is normally stored and rapidly secreted after injury and identify both upstream and downstream associated growth factor pathways, thereby providing additional insight into the orchestrated activation of transcriptional and signaling pathways in vascular disease.1

The authors found that ATF-4 is minimally expressed at baseline, is rapidly induced in the carotid 4 hours after injury, and persists at lower expression levels at 5 and 14 days after injury, as measured by immunohistochemistry. In vitro, cultured rat SMCs subjected to scraping injury also demonstrated increased ATF-4 expression within 2 hours after injury. To confirm a primary role for FGF-2 in ATF-4 induction, the authors subsequently treated cultured SMCs with FGF-2 and verified that ATF-4 protein is induced within 2 hours of treatment. These findings provide, for the first time, evidence that ATF-4 is an important mediator of intimal thickening after vascular injury.

ATF-4 is a signal-responsive member of the basic leucine zipper family of transcription factors, binds to the cAMP response element, and is of particular interest because of its activation both by transcriptional and translational mechanisms in response to various forms of cellular stress. ATF-4 has been shown previously to be induced by hypoxia and amino acid deficiency and in response to endoplasmic reticulum (ER) stress. Until the present study by Malabanan et al,1 ATF-4 has not been implicated previously in vascular disease. Many transcription factors have been implicated in the pathogenesis of occlusive vascular lesions; however, the link between upstream activators and downstream effectors has been less well characterized. The study by Malabanan is distinguished from other studies in that it goes beyond simple induction of ATF-4 by injury and FGF-2 and identifies potentially important signaling pathways downstream of FGF-2 responsible for ATF-4 induction. Through the use of inhibitors of the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase pathways, the authors determine that FGF-2 activation of PI3K is required for induction of ATF-4, which correlates with phosphorylation of AKT, the major substrate for PI3K. Inhibition of mitogen-activated protein kinase, in contrast, had no effect. Furthermore, the authors provide novel data indicating that ATF-4 activates vascular endothelial growth factor (VEGF)-A expression in response to FGF-2.

To demonstrate activation of VEGF-A gene expression by ATF-4, the authors used multiple approaches. Induction of VEGF-A has previously been described in vascular injury models and in SMCs after FGF stimulation; however, the transcriptional mechanisms have not been fully elucidated. The authors found that overexpression of ATF-4 in SMCs enhanced expression of endogenous VEGF-A mRNA levels. Furthermore, both FGF-2 and scraping injury induced ATF-4 binding activity in nuclear extracts, as measured by electrophoretic mobility-shift assays using an ATF-4-binding site oligonucleotide derived from the VEGF-A promoter. These findings were further confirmed through the use of chromatin...
immunoprecipitation in FGF-2–treated cells, in which ATF-4 antibody was shown to immunoprecipitate the ATF-4 binding site of the VEGF promoter. The authors also mutated the ATF-4–binding sites in VEGF–luciferase reporter constructs and showed that FGF-2 induction of reporter activity was abolished when compared to wild-type constructs and that fibroblasts lacking ATF-4 did not demonstrate FGF-2–induced induction of VEGF-A when compared to wild-type fibroblasts. Finally, the authors used ATF-4 small interfering RNA to inhibit ATF-4 induction, VEGF-A induction, and neointimal formation after vascular injury, further underscoring the intimate relationship between ATF-4, VEGF-A, and intimal hyperplasia.

Although the study by Malabanan et al1 provides strong evidence for a role for ATF-4 in vascular injury, a number of important questions remain. Given the complexity of the process of neointimal formation and the pleiotropic effects of growth factors and transcription factors in cellular physiology, it is difficult to imagine that a simple model involving linear activation of FGF-2, ATF-4, and VEGF-A can fully explain the pathogenesis of vascular injury. Because the authors initially identified ATF-4 as an FGF-2 target in a microarray screen, one can hypothesize that a more complete bioinformatic analysis of FGF-2 target genes that are activated in SMCs would yield a more comprehensive view of transcriptional pathways activated downstream of FGF-2. Bioinformatic techniques such as Gene Set Enrichment Analysis11 would be useful to obtain such a global evaluation. Identification of important growth factor–dependent pathways in vitro may also provide further insights into the relevant important pathways in vivo.

Additionally, ATF-4 also has been linked to a variety of cellular responses and has numerous identified target genes. The data presented by Malabanan et al1 do not address whether there are additional targets of ATF-4 in addition to VEGF-A that may be contributing to smooth muscle proliferation and intimal thickening after injury. The authors speculate that ATF-4 may be regulating other relevant molecules such as platelet-derived growth factor receptor-α, cholesterol 25-hydroxyrase, and the angiotensin II receptor; however, a comprehensive and systematic evaluation of ATF-4–dependent target genes in SMCs would ultimately provide additional useful information.

One particular aspect of ATF-4 biology that is intriguing and worthy of further study involves the relationship to ER stress, also referred to as the unfolded protein response (reviewed elsewhere2,13). ATF-4 functions downstream of PERK (protein kinase RNA-like ER kinase) and is activated by increased phosphorylation of eukaryotic initiation factor-2α, which leads to increased translation of ATF-4 mRNA into protein. To the authors’ credit, they present additional data that vascular injury and FGF-2 treatment lead to increased phosphorylation of eukaryotic initiation factor-2α, which implies that ER stress is an important mechanism in vascular injury and may also contribute to the induction of ATF-4 protein expression. ATF-4 is known to increase the expression of other stress response genes that promote recovery, such as the chaperone Grp78,7 genes involved in amino acid import, glutathione synthesis, and resistance to oxidative stress.14 Future work detailing the downstream targets of ATF-4 relevant to ER stress will be of significant interest.

Another important question raised by the study of Malabanan et al1 concerns the role of VEGF-A in vascular injury and smooth muscle proliferation. Although the data presented are consistent with a role for VEGF-A in promoting intimal hyperplasia and are also consistent with previously published in vivo studies,9–18 others have reported the contradictory finding that VEGF suppresses intimal hyperplasia through promotion of reendothelialization.19,20 The authors appropriately point out this contradiction, which will await future studies for clarification.

The study by Malabanan et al1 is significant in that it links vascular injury to a transcription factor, ATF-4, associated with cellular stress responses and assesses both upstream (FGF-2) and downstream growth factor pathways (VEGF-A) that affect or are affected by expression of ATF-4 in response to vascular injury. Although the study raises many additional questions about the relationship of ATF-4 to other growth factor signaling and transcriptional pathways linked to vascular injury, it provides a clear framework for future investigation. Overall, it provides important insights into a complex process by demonstrating that ATF-4 functions as an integrator of the FGF-2 signal and an activator of the VEGF signal, thereby bridging 2 significant growth factor pathways, while orchestrating the stress response of the injured smooth muscle cell.

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