The transcriptional response to vascular injury. The vascular response to injury is a dynamic and multifactorial process involving several cell types. As part of the vascular response to injury, normally quiescent, contractile vascular smooth muscle cells (VSMCs) respond to inflammatory and growth factors by transforming from a differentiated contractile state to a dedifferentiated synthetic phenotype capable of migration, proliferation, and synthesis of cytokines and extracellular matrix. As the major effector cell in this occlusive process, the VSMC must coordinate and synchronize immensely complex inflammatory, proliferative, and differentiation programs. Indeed, the activated “myofibroblastic” VSMCs can even express proteins and cytokines ascribed to be restricted to inflammatory cells. Several transcription factors have earned the moniker of “master switch” of these processes?

Kruppel Complexity

The Krüppel-like family of transcription factors contain zinc finger DNA binding domains and presently include at least 16 mammalian members, with several having important roles in cardiovascular pathophysiology. One might think that there would be redundancy among so many family members; however, the non–zinc finger domain of these transcription factors displays structural heterogeneity, resulting in surprising functional diversity among Krüppel proteins. In particular, the role of Krüppel-like factor (KLF)4 in vascular biology and the mechanisms of its effects are as complex as the cells that participate in vascular disease are diverse.

For example, in macrophages, KLF4 is induced by inflammatory stimuli and mediates induction of proinflammatory genes by 2 different, but complementary, mechanisms. In the first, KLF4 interacts with the NF-κB subunit p65 and cooperates with its transactivation of proinflammatory genes. In the other, KLF4 competes with Smad3, a mediator of transforming growth factor-β1 antiinflammatory signaling, for its coactivator, p300, resulting in inhibition of antiinflammatory gene expression. In endothelial cells (ECs), KLF4 is also induced by inflammatory stimuli and disturbances in laminar flow but with antiinflammatory effects. KLF4 exerts a protective, antiinflammatory phenotype in these cells also by binding to NF-κB subunits, but in ECs, this leads to an inhibition of NF-κB–mediated inflammatory gene expression. KLF4 also directly transactivates expression of antiinflammatory genes in ECs such as endothelial NO synthase.

KLF4 is not normally expressed in VSMCs in uninjured arteries but is transiently induced in rat carotid arteries by balloon angioplasty, where it functions in at least 2 ways. KLF4 participates in growth arrest of VSMCs by enhancement of p53 expression, and subsequent increase in 1 of its target genes p21WAF/Cip1, a potent suppressor of cell cycle progression. In VSMCs, KLF4 also drives phenotypic switching by suppression of smooth muscle cell (SMC) marker genes. KLF4 can bind the transforming growth factor-β control element in the promoter of SMC differentiation genes and interact with serum response factor (SRF), preventing SRF from associating with promoters of SMC differentiation genes. KLF4 can also repress expression of the SRF coactivator myocardin. Clearly, KLF4 expression and activity has profound pleiotropic effects on multiple cell types that participate in vascular pathophysiology. Unfortunately, its effects in vivo have been understudied because of limited viability of KLF4 knockout (KO) mice.

KLF4 Regulates the Vascular Response to Injury by Multiple Mechanisms

In this issue of Circulation Research, Yoshida et al16 circumvent lethality issues by using a tamoxifen-inducible KLF4 conditional deletion approach and clarify the role of this transcription factor in the in vivo VSMC response to injury. This work combines multiple in vivo and mechanistic approaches to address the function of KLF4 in regulation of intimal hyperplasia and is the first to report that abrogation of KLF4 enhanced development of neointima in ligation injured mice.

In wild-type mice, KLF4 mRNA is rapidly and transiently expressed in response to ligation injury, which immediately preceded a decrease in the differentiation marker smooth muscle (SM)α actin mRNA expression. However, in KLF4 KO mice, this differentiation marker repression was delayed but not abrogated. Although not addressed by the authors, this suggests that other transcription regulators can compensate for the loss of KLF4.
for KLF4; potential candidates for this other factor might be KLF5, because it can use the SM22α promoter in reporter assays, or KLF13 and/or KLF15, both expressed in SMCs and associated with the SM22α promoter.

One mechanism for the delay in repression of differentiation markers might be that chromatin immunoprecipitation analysis in lysates from ligated mouse carotid arteries determined that KLF4 bound to the transforming growth factor-β control element in the SMα actin and SM22α promoters. Medial cells in KLF4 KO mice demonstrated enhanced proliferation compared with control mice. This is surprising, and somewhat counterintuitive, considering the context of prolonged expression of differentiation markers in these KO mice.

Prior studies indicated a strong relationship between KLF4 and expression of the p53 tumor suppressor. One target of p53 is p21\(^{\text{WAF/Cip1}}\), a potent negative regulator of cell cycle progression. Yoshida et al, provide multiple lines of mechanistic evidence to support the notion that a likely mechanism of KLF4 effects on growth regulation is that p21\(^{\text{WAF/Cip1}}\) expression is not increased by ligation injury in KLF4 KO mice, whereas forced KLF4 expression induced p21\(^{\text{WAF/Cip1}}\) protein expression in cultured VSMCs. Furthermore, KLF4 binding sites were identified in the p21\(^{\text{WAF/Cip1}}\) promoter, and KLF4 regulated p21\(^{\text{WAF/Cip1}}\) transactivation in a p53-dependent manner.

Progression of intimal hyperplasia is initiated and sustained by the inflammatory response. One limitation to this study is that it cannot definitively be determined whether the inflammatory response was altered in these mice. The authors do report that inflammatory cell infiltration into the ligated artery was no different between KLF4 KO and wild-type mice, but nothing is known about the function of these KLF4-null inflammatory cells. KLF4 induces and regulates expression of inflammatory factors in macrophages, so one might expect reduced inflammation in injured arteries of these mice if they do not synthesize the appropriate repertoire of inflammatory factors. This would support the assertion by the authors that these proliferative effects are directly attributable to modulation of SMC gene transcription. On the other hand, we know ECs occupy a critical role in maintenance of the quiescent phenotype of VSMCs. In ECs, KLF4 expres-
sion is increased by shear stress, leading to increased expression of antiinflammatory factors, which would be expected in the ligated murine carotid arteries used in this study. It needs to be considered that lack of antiinflammatory signals from ECs could indirectly affect VSMC phenotype and contribute to increased neointima formation in vivo. As the authors appropriately point out in their discussion, cell-specific deletion of the klf4 gene is necessary to define the precise role of this protein in each of these cell types to fully characterize the role of this transcription factor in so dynamic a process development of intimal hyperplasia.

**Perspective**

One can envision an in vivo scenario in which activated macrophages secrete inflammatory factors mediated by KLF4 expression, which induce ECs to assume an antiinflammatory, nonthrombogenic phenotype, also mediated by KLF4, which, in turn, stimulates KLF4 in SMCs, inducing an antiproliferative, yet dedifferentiated phenotype (Figure).

In a larger sense, the seemingly paradoxical finding that differentiated SMCs can proliferate is perhaps the most important contribution by Yoshida et al and implicates KLF4 as a transcriptional link between proliferation and differentiation, at least in SMCs. However, it is not the end of the story, and, like most important studies, the report points out that additional studies necessary to determine the role of KLF4 in regulation of inflammatory gene expression in VSMCs. Like other zinc finger–containing proteins, the activity of the Kruppel proteins can be regulated not only by de novo synthesis but also by posttranslational modification and protein–protein interactions. How these are influenced by extracellular stimuli, both autocrine and paracrine, from other cell types remains to be elucidated.

Identification and characterization of transcriptional regulators of intimal hyperplasia are not only crucial in our better understanding of this dynamic process but also represent potential targets for interventional modalities. KLF4 may indeed fit the bill as a single transcription factor that can play a central role in regulation of proliferation, or inflammation, or differentiation, and all 3.

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None.

**References**


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Krüppel-Like Factor 4: Transcriptional Regulator of Proliferation, or Inflammation, or Differentiation, or All Three?
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