

Endothelial KLF4: Crippling Vascular Injury?

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The health of the vascular endothelium is critical for normal vascular physiologic function, and dysfunction of the endothelium can lead to development of vascular disease, such as atherosclerosis and thrombosis. Endothelial cells (ECs) integrate hemodynamic and biochemical signals to regulate principal vascular functions, including vasoreactivity, permeability, immune cell homing, and thrombosis. Molecular, cellular, and genetic approaches over the past decade have strongly implicated members of the Krüppel-like factor (KLF) family of transcription factors as essential regulators of endothelial function in health and disease. *Krüppel* is the German word for “cripple,” a name given to the gap gene identified in *Drosophila* embryos whose absence results in abnormal segmentation and death.¹ In this issue of *Journal of American Heart Association*, Yoshida and colleagues add important insights to this field and identify endothelial KLF4 as a negative regulator of neointimal formation in the biological response to vascular injury by counteracting the actions of nuclear factor kappa B (NF- κ B), a central regulator of inflammation.²

The development of methods to culture ECs in the early 1970s was a major advance that ushered in the modern era of vascular biology research.^{3,4} Subsequent work from many laboratories has revealed that these cells can alter their phenotype in response to various physiologic and pathologic stimuli. For example, stimulation of the endothelium with proinflammatory cytokines produces a dysfunctional endothelium associated with elaboration of proadhesive and -thrombotic factors.^{5,6} Conversely, laminar flow induces an

opposing gene profile associated with antithrombotic, -adhesive, and -inflammatory properties.^{7,8} Importantly, human arterial segments subject to laminar flow are less likely to develop atherogenic lesions, in contrast to “atheroprone” arterial branch points exposed to nonlaminar or disturbed flow.⁹ Emphasis on identifying central regulators integrating the effects of both hemodynamic and biochemical stimuli in the endothelium led to the discovery of the KLF family of transcription factors. Though several members of this family have been implicated in endothelial biology, the data are most compelling for KLF2 and KLF4.^{10–12}

Initial links between KLFs and the endothelium were gleaned from the observations of Kuo and colleagues, who found that within embryonic vascular tissues, KLF2 was principally expressed in the endothelium.¹¹ Subsequent work from several laboratories identified KLF2 and KLF4 as laminar flow-inducible transcription factors that broadly regulate endothelial gene products governing vasoreactivity, permeability, immune cell adhesion, and thrombosis.⁹ Furthermore, both factors were shown to inhibit endothelial proinflammatory targets by virtue of their ability to antagonize NF- κ B.^{13,14} Whereas in vitro functional studies were highly supportive of the notion that KLF2 and KLF4 controlled basic endothelial functions, in vivo confirmation proved to be more difficult until recently.^{13,15} This gap in physiologic relevance was the result, in large part, of the challenges associated with the viability of KLF2 and KLF4 knockout (KO) mice.^{11,16} For example, global or endothelial-specific deletion of KLF2 results in death during embryonic development.¹¹ Furthermore, global deletion of KLF4 results in death within hours of birth, thereby precluding studies in adult animals.¹⁶ However, endothelial-specific KLF4 KOs are viable.¹⁷ Using this mouse line and a second line overexpressing KLF4 in the endothelium, Zhou and colleagues showed recently that KLF4 confers an antiatherogenic and -thrombotic phenotype.¹⁷ Mechanistically, these effects were linked to the ability of KLF4 to inhibit NF- κ B activity by affecting recruitment of critical coactivators to induce target genes, such as vascular cell adhesion molecule 1 (VCAM-1).¹⁷ Collectively, these observations provided the most cogent in vivo evidence to date implicating endothelial KLFs as essential regulators of vascular function in the adult animal.

The current study by Yoshida and colleagues builds on the aforementioned work and evaluates the role of endothelial

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KLF4 in a model of vascular remodeling.² Using the genetic background of Tie2-Cre and KLF4^{loxP} mice, the authors generated endothelial-specific deletion of KLF4. Mice subjected to carotid artery ligation demonstrated enhanced neointimal formation postinjury, a finding attributed to enhanced vascular smooth muscle cell (VSMC) proliferation and recruitment of inflammatory cells (macrophages and T lymphocytes). Importantly, KLF4 expression was induced within the VSMC compartment of both wild-type and EC-KLF4-deficient mice in response to injury, and smooth muscle cell (SMC) differentiation marker expression (smooth muscle [SM] 22 α and SM-actin) was no different, thereby strongly implicating the contribution of endothelial KLF4 to this altered phenotype. By day 21, EC-KLF4-deficient mice exhibited greater neointimal formation, as compared to controls. Examination of injured tissue revealed enhanced endothelial expression of cell adhesion markers in EC-specific KLF4 KO mice. The researchers explain that the greater VCAM-1 and E-selectin expression likely resulted in the enhanced recruitment and accumulation of inflammatory cells in the neointima. Mechanistic studies showed that tumor necrosis factor alpha-induced expression of VCAM-1 is augmented in the absence of KLF4. Detailed VCAM-1 promoter analysis revealed the presence of one KLF4 and two NF- κ B consensus-binding sites. Furthermore, chromatin immunoprecipitation studies revealed that the direct binding of KLF4 to p65 in human umbilical vein endothelial cells transduced with KLF4 inhibited NF- κ B activity at the VCAM-1 promoter in response to inflammatory stimuli. Interestingly, Yoshida and colleagues previously reported enhanced neointimal formation in KLF4 conditional KO mice after vascular injury resulting from reduced induction of p21^{WAF1/CIP1}, a potent inhibitor of cell cycle progression.¹⁸ They identified KLF4-binding sites in its promoter and showed that KLF4 regulated p21^{WAF1/CIP1} expression in a p53-dependent manner.¹⁸ Taken together, the current and previous findings identify endothelial KLF4 as a regulator of vascular injury-mediated neointimal hyperplasia through anti-inflammatory and -proliferative effects (Figure).

From a historical perspective, Forrester and coworkers proposed a paradigm for neointimal hyperplasia as a general wound-healing response based largely on observations from animal studies.¹⁹ Endothelial denudation, platelet deposition, inflammatory cell infiltration, release of growth factors, medial SMC modulation and proliferation, proteoglycan deposition, and extracellular matrix remodeling were identified as the major milestones in the temporal sequence of this response. The centrality of inflammation in this response was proposed initially by Dr. Peter Libby in 1992,²⁰ and a series of studies by our laboratory demonstrated that leukocyte recruitment and accumulation, mediated by the leukocyte integrin, macrophage-1 antigen, and the platelet counter-receptor,

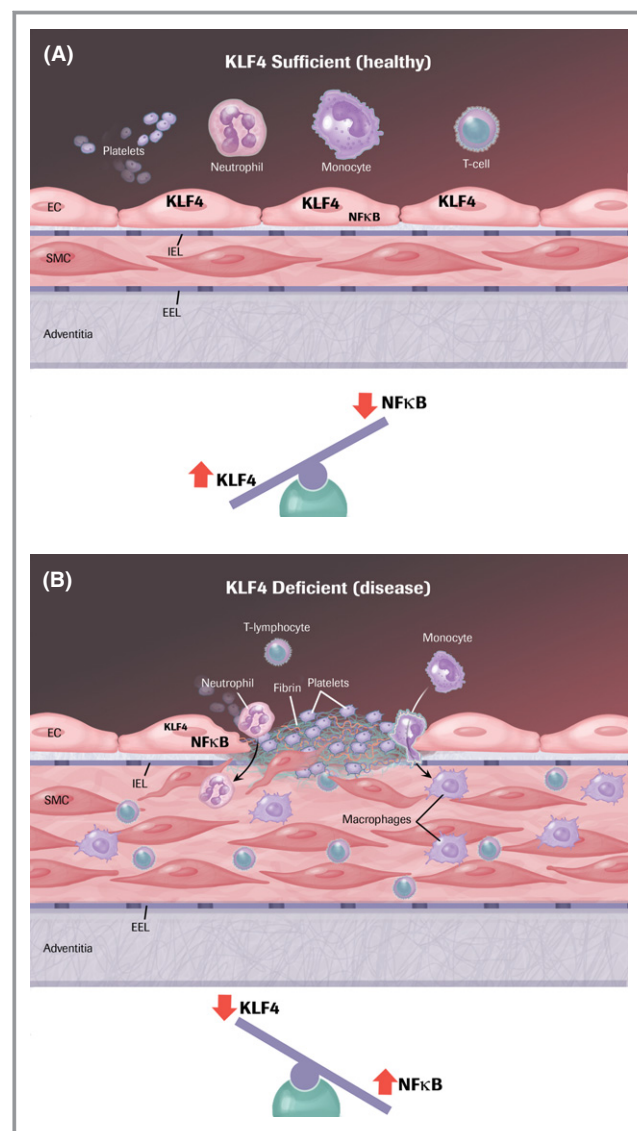


Figure. Diametric relationship of KLF4 and NF- κ B affects vascular endothelial health. Schematic diagram representing antithetical expression of KLF4 and NF- κ B in endothelial cells. A, KLF4 presence and diminished NF- κ B activity promotes vascular integrity. B, KLF4 absence and enhanced NF- κ B activity facilitate injury-mediated vascular disease associated with inflammatory cell recruitment and neointimal hyperplasia. EC indicates endothelial cell; EEL, external elastic lamina; IEL, internal elastic lamina; KLF4, Krüppel-like factor 4; NF- κ B, nuclear factor kappa B; SMC, smooth muscle cell.

glycoprotein Iba α , drives the neointimal hyperplasia response.^{21,22} In both balloon-angioplastied and stented arteries, re-endothelialization of at least part of the injured vessel surface occurs, and accelerating EC coverage attenuates neointimal formation.²³ The importance of the endothelium in neointimal formation is vascular injury and model dependent. Animal models of neointimal hyperplasia that preserve the endothelium (eg, carotid ligation, perivascular cuff) are mechanistically distinct from endothelial

denuding animal models (eg, air dry, wire injury) and clinical restenosis (eg, balloon angioplasty and stenting).

Although the Yoshida and coworkers nicely characterize the role of endothelial KLF4 in the carotid artery ligation model of vascular injury that largely preserves the endothelium,² several limitations merit consideration. The Tie2 promoter exhibits activity in cells of the hematopoietic compartment. Importantly, KLF4 confers anti-inflammatory effects in myeloid cells, and recently, our group demonstrated that KLF4 myeloid deficiency results in enhanced inflammatory cell infiltration of atherosclerotic lesions.²⁴ Although the researchers demonstrate no significant difference between KLF4 levels in peripheral blood of control and EC-specific KLF4 KO mice,² the presence of KLF4-sufficient cellular contaminants cannot be excluded. Therefore, KLF4-deficient monocytes and macrophages may be contributing to the observed phenotype of enhanced neointimal formation of Tie2-Cre-derived EC-specific KLF4 KO mice. Furthermore, the contribution of additional factors regulated by KLF4, such as nitric oxide, cytokines/chemokines, and matrix-modifying enzymes, that may play a role in the observed phenotype were not presented. Several key questions are evident. First, if KLF deficiency promotes vascular disease, what are the upstream regulators of KLF4? Second, does KLF4 regulate EC proliferation and migration that might influence re-endothelialization after vascular injury? Third, what signals mediate the crosstalk between ECs and VSMCs that drives an increase in VSMC KLF4 expression and subsequent VSMC proliferation after injury? Fourth, is KLF4 deficiency responsible for premature atherosclerosis in diabetic patients as well as others with accelerated vascular disease (eg, HIV infection, transplant vasculopathy)? Finally, it would be of interest to know whether sustained overexpression of endothelial KLF4 abrogates the effects of vascular injury. Because KLF4 deficiency has been linked to atherothrombosis,¹⁷ pulmonary vascular disease,²⁵ and, currently, restenosis, could boosting KLF4 expression be therapeutically beneficial in patients with vascular disease? Interestingly, statins have been shown to enhance endothelial KLF4 expression, providing a nonlipid drug benefit derived from KLF4 activity in the endothelium.²⁶ Also, recently, an abciximab-KLF4 expression plasmid dual-delivery intracoronary stent was shown to maintain intraluminal area and reduce in-stent restenosis in a porcine model of coronary injury.²⁷

Collectively, despite its limitations, the findings of this article support an important role for KLF4 in the maintenance of vascular health and its deficiency in aggravating vascular disease. Targeting transcription factors has largely remained elusive in human disease. Further insights into upstream factors that regulate KLF4 expression as well as new therapeutic approaches (eg, RNA interference, chemical epigenetic regulators) might provide new avenues for mod-

ulating neointimal formation. Understanding how to safely and efficiently translate these experimental observations might lead to new clinical applications, perhaps targeting KLF4.

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Disclosures

None.

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