Role of Krüppel-Like Transcription Factors in Endothelial Biology

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Abstract—Krüppel-like factors are members of the zinc finger family of transcription factors that have been implicated as playing key roles in regulating cellular differentiation and tissue development. Studies over the past several years support an important role for this family of factors in vascular biology. This review summarizes the role of Krüppel-like factors in endothelial cell biology. (Circ Res. 2007;100:1686-1695.)

Key Words: endothelium ▪ transcription ▪ vessels ▪ gene expression ▪ gene regulation

The vascular endothelium is a vital organ whose health is essential to normal vascular physiology and whose dysfunction can be a critical factor in the pathogenesis of vascular disease.1-3 Clinical, pathological, and experimental observations support a central role for endothelial cell dysfunction in the development of vascular disease states such as atherosclerosis and thrombosis.4-6 Endothelial cells lining the blood vessels are critical integrators and transducers of various physiological stimuli. They are actively involved in many physiological processes, such as regulation of selective permeability, blood coagulation, and homing of immune cells to specific sites of the body.5 Various stimuli can modulate the phenotype of endothelial cells. For example, laminar flow induces factors such as endothelial nitric oxide synthase (eNOS) and thrombomodulin (TM) that confer potent anti-thrombotic, antiadhesive, and antiinflammatory properties to the endothelium.7,8 Conversely, a number of noxious stimuli, such as turbulent flow, proinflammatory cytokines, or advanced glycation end products (seen in diabetic patients),9 can render the endothelium dysfunctional. As a consequence, eNOS expression is reduced, adhesion molecule expression is induced (eg, vascular cell adhesion molecule [VCAM]-1), and a procoagulant phenotype is observed.5,10,11

Pathologic studies demonstrate that the earliest lesions in atherosclerosis occur in a nonrandom fashion at branch points and areas of major curvature.2,3 These observations led to the hypothesis that local events and/or intrinsic differences in the endothelial cells at specific regions of the vascular tree may contribute to the focal nature of vascular disease. Accumulating evidence suggests various forms of shear stress can differentially modulate various aspects of endothelial cell function.7 Blood flow patterns can vary in complexity from the relatively uniform laminar shear stress (seen in unbranched portions of vessels) to complex disturbed or turbulent flow patterns (seen near branch points and major curves).3,12,13 Laminar shear stress has been shown to confer antithrombotic, antiadhesive, and antiinflammatory effects as well as enhance endothelial survival.5,14 This occurs through the induction of certain gene products such as eNOS.7 Over the last several years,
there has been a considerable amount of research implicating Krüppel-like transcription factors as key regulators of the endothelial pathways discussed above. This review will focus on the role of Krüppel-like factors (KLFs) in endothelial biology.

The Krüppel-Like Factor Family of Transcription Factors

KLFs are a subclass of the zinc finger family of DNA-binding transcription factors. The nomenclature is based on the homology to the DNA-binding domain of the *Drosophila* protein Krüppel. Krüppel is the German word for “cripple.” The protein is aptly named because *Drosophila* embryos homozygous for the protein Krüppel have altered anterior abdominal and thoracic segments, resulting in death. The first mammalian Krüppel, erythroid Krüppel-like factor (EKLF/KLF1), was identified in red blood cells. Since the initial discovery of EKLF in 1993, a total of 17 mammalian Krüppels have been identified and designated based on the chronological order of discovery (ie, KLF1 to -17).

Several features distinguish KLFs from other members of the zinc finger family of transcription factors. The C terminus of KLF proteins contain 3 Cys2/His2 zinc fingers. The interfinger space sequence contains a highly conserved 7-aa sequence, TGEKP(Y/F)X. Many KLFs are able to bind to similar DNA sequences, such as the “CACCC” sequence or the “GT box.” One explanation for this similar binding came from structural studies revealing that DNA-binding specificity is dictated by 3 critical residues within each zinc finger. Although the zinc finger domains are very similar, the non–DNA-binding domains are highly divergent. Transcriptional regulation by KLFs is mediated via their identified modular activation and repression domains (Figure 1).

KLFs have been identified as playing key roles in regulating cellular processes in many distinct cell types (reviewed previously). To date, 3 members of the KLF family have been reported as being expressed in endothelial cells: KLF2, KLF4, and KLF6 (Figure 1). This review discusses published studies that implicate these KLFs as playing important roles in endothelial biology.

**Figure 1.** Schematic comparison of subdomains for selected KLFs discussed in this review. These domains are based on work by Conkright et al (KLF2), Yet et al (KLF4), and Ratziu et al (KLF6). ZN* indicates Zn²⁺.

Krüppel-Like Factor 2: Identification and Characterization

KLF2 was first cloned by Lingrel and colleagues, with a homology-based approach that used the zinc finger domain of EKLF/KLF1. KLF2 is a 354-aa protein that, owing to its high expression in lung tissues, was initially termed lung Krüppel-like factor (LKL). Human KLF2 maps to chromosome 10p13.1 and has greater than 85% homology to the mouse gene. Furthermore, critical regions of the promoter are also conserved across species, including exact identity of a 75-bp sequence. Crude structural domain mapping experiments have identified an N-terminal transcriptional activation domain between amino acids 1 and 110 and an inhibitory domain between amino acids 110 and 267 (Figure 1). This inhibitory domain has been shown to interact with the WW domain–containing E3 ubiquitin protein ligase 1 (WWP1), resulting in ubiquitination and proteasomal degradation of KLF2.

KLF2 expression is developmentally regulated, with expression in mouse embryos at embryonic day 7 (E7), decreased expression at E11, and a subsequent increase at E15. Targeted deletion analysis revealed that KLF2 is an important regulator of T-cell maturation, programming single positive T-cell quiescence. Similar conclusions were obtained using forced expression studies in T cells. More recent data also implicate KLF2 as playing a critical role in thymocyte and T-cell trafficking.

KLF2 has also been determined to be essential for normal lung development. As KLF2 knockout results in embryonic lethality (discussed in more detail below), Wani and colleagues constructed KLF2-null homozygous embryonic stem cells to generate chimeric mice and determine whether these cells could give rise to normal lung tissue. The authors demonstrate that in chimeric animals that survived after birth, null embryonic stem cells contribute significantly to all of the major organs except the lungs. In contrast, histopathologic studies on the chimeric animals that died at birth revealed abnormalities in their lung development. Taken together, these observations identify KLF2 as requisite for normal lung development.

Finally, we note that KLF2 deficiency is lethal, as mouse embryos do not survive past E12.5 to E14.5 because of intraembryonic and intraamniotic hemorrhage. Surpris-
ingly, despite the fact that KLF2 expression is limited to endothelial cells within the vessel wall, vasculogenesis and angiogenesis have been reported as being unaffected in these animals. However, the KLF2 knockout mouse exhibits impaired blood vessel maturation caused by the lack of smooth muscle cell recruitment and tunica media formation, resulting in aneurysmal dilation of both arteries and veins and subsequent rupture.29 More recent data, using tissue specific KLF2 knockout mouse models, demonstrate that endothelial loss of KLF2 results in lethal embryonic heart failure attributable to a high-cardiac-output state.40 Further analysis reveals that the cause of high-cardiac-output heart failure is not a result of anemia or atriovenous malformations but rather the result of a profound loss of peripheral vascular resistance. Lethal high-output heart failure can be rescued by using the catecholamine phenylephrine to increase vessel tone.40 The mechanism by which KLF2 regulates vascular tone and the stability of the blood vessel wall during embryonic development remains poorly understood.

Similar to rodent data, KLF2 is also expressed in the endothelial lining of human vessels. Using in situ hybridization approaches, Horrevoets and colleagues showed that KLF2 expression is limited to the endothelial layer of the human aorta. Importantly, these investigators also showed that KLF2 expression is decreased at branch points.41 This finding is intriguing, as pathologic studies have identified arterial branch points as the earliest atheroprone regions of the human vasculature.2,3 Over the last several years, there has been a flurry of research conducted on KLF2 and its role in endothelial cell function, making it the most widely studied and published KLF in endothelial cells. Below we outline the data that implicate KFL2 as a key “molecular switch” that regulates important aspects of vascular function and disease (Figure 2).

**Functions of KLF2 in Endothelial Biology**

Regulates Leukocyte Adhesion to the Endothelium

Endothelial cells respond to inflammatory stimuli by inducing the expression of chemokines and adhesion molecules that recruit immune cells to the blood vessel wall. This early event sets the stage for a series of complex interactions between these immune cells and nonimmune cells that dictate disease development and progression.6 Studies from our laboratory were the first to implicate KLF2 as a key transcriptional regulator of endothelial proinflammatory activation. In this study, we observed that KLF2 potently inhibits cytokine-mediated induction of VCAM-1 and E-selectin expression, resulting in decreased immune cell attachment and

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**Figure 2.** Schematic diagram of the regulation and function of KLF2 in endothelial cells. APC indicates activated protein C; ASS, argininosuccinate synthase; CNP, C-natriuretic peptide; ET-1, endothelin 1; SEMA3F, semaphorin-3F; TF, tissue factor; tPA, tissue plasminogen activator.

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**Summary of key KLF2 targets**

- α-inflammatotry: VCAM-1, E-selectin, eNOS
- α-thrombotic: PAI-1, TF, TM, eNOS, tPA
- Vasodilatory: ET-1, CNP, eNOS, ASS
- α-angiogenic: VEGFR2, SEMA3F
rolling to an endothelial monolayer. There was no significant inhibition of intercellular adhesion molecule (ICAM)-1 expression, demonstrating the specificity of this effect.42

Interestingly, KLF2 inhibits endothelial cell activation by diverse proinflammatory stimuli, including interleukin (IL)-1β, tumor necrosis factor (TNF)α, lipopolysaccharide, and thrombin,52,43 suggesting that some common proinflammatory pathway may be affected by KLF2 action. Among the most important proinflammatory pathways implicated in endothelial proinflammatory activation is that of nuclear factor (NF)-κB. NF-κB is composed of homo- or heterodimeric complexes of members of the Rel family of proteins, consisting of p65 (RelA), c-Rel, RelB, p50, and p52. The best studied and most abundant of these complexes is the p50/p65 heterodimer (NF-κB). Normally, in the absence of inflammatory stimuli, NF-κB is maintained in the cytoplasm so as to prevent activation of gene transcription indiscriminately by 2 classes of inhibitors: the IκB molecules and the p105/p100 proteins. The former molecules bind only to NF-κB and maintain it in the cytoplasm. The latter group serves as both inhibitors and as uncleaved precursors of the NF-κB DNA-binding subunits, p50 and p52. On cellular stimulation, the IκB kinase (IKK) complex becomes active and phosphorylates the inhibitors, resulting in the complete degradation of IκB inhibitors, or partial degradation of p100/p105. As a consequence, NF-κB dimers (principally p50/p65) are formed, which then move into the nucleus to affect gene expression. Optimal NF-κB activity is also dependent on a number of coactivator proteins including CREB-binding protein (CBP) and its structural homolog p300, steroid receptor-coactivator-1 (SRC-1), IKKα, and p300/CBP-associated factor.44–50

Indeed, we determined the mechanism of the inhibition of endothelial cell activation by KLF2 to be through inhibition of NF-κB function at multiple levels. In the case of IL-1β, KLF2 does not affect the expression of any of the components of the NF-κB pathway. Interestingly, KLF2 does not alter p50/p65 nuclear accumulation or DNA-binding. However, KLF2 does interact with CBP/p300, a key cofactor required for optimal NF-κB activity. By competing with NF-κB for this transcriptional coactivator, KLF2 reduces NF-κB-dependent transcriptional activity and expression of target genes such as VCAM-1 and E-selectin in response to inflammatory cytokines (Figure 2).42 In contrast, another mechanism is operative for thrombin. Thrombin-mediated induction of multiple cytokine/chemokines, including IL-6, IL-8, and monocyte chemotactrant protein-1 (MCP-1), is inhibited by KLF2 through the ability of KLF2 to inhibit protease-activated receptor 1 (PAR-1), the principal receptor of thrombin. This inhibition of PAR-1 results in decreased nuclear accumulation and subsequent DNA binding of NF-κB.43 Thus, the ability of KLF2 to inhibit endothelial inflammation occurs via multiple distinct mechanism that inhibit the NF-κB pathway. These initial studies have been subsequently verified by genomic profiling studies by our group and others.51,52

More recently, studies by Horrevoets and colleagues have implicated KLF2 as playing a role in the activating protein 1 (AP-1) pathway.53,54 Boon et al53 demonstrate that KLF2 strongly inhibits transforming growth factor (TGF)-β signaling via 2 distinct mechanisms. Using overexpression and knockdown studies, they show that KLF2 induces Smad7, subsequently suppressing Smad2 phosphorylation and Smad3/4-dependent transcriptional activation. In addition, KLF2 simultaneously inhibits the TGF-β signaling cofactor AP-1. Fledderus et al44 go on to demonstrate that human endothelial cells overlying atherosclerotic plaques exhibit increased levels of phosphorylated nuclear activating transcription factor 2 (ATF2), which is among the heterodomeric components of AP-1. Using knockdown and overexpression studies, they further show that shear stress, via KLF2, suppresses nuclear levels of activated ATF2 by inhibiting its nuclear translocation (Figure 2).

Regulates Endothelial Thrombotic Function

Under physiologic conditions, the vascular endothelium maintains blood fluidity through the production of factors that promote fibrinolysis (eg, tissue plasminogen activator), inhibit blood coagulation (eg, TM), and inhibit platelet activation (eg, NO).55,56 If the capacity of the endogenous anticoagulation systems is overwhelmed by a noxious stimulus or the capacity of inhibitory pathways is impaired, unwanted hemostasis may occur.56,57 Studies by Lin et al demonstrated that KLF2 can differentially regulate key factors involved in maintaining an antithrombotic endothelial surface. KLF2 can potently induce TM, a cell surface factor involved in the generation of activated protein C through its interactions with thrombin. Mechanistic studies demonstrated that KLF2 can bind to specific regions within the TM promoter to induce expression. Thus, by inducing TM expression, KLF2 can augment antithrombotic activity via the conversion of protein C to activated protein C (Figure 2).58 In addition, the cytokine-mediated activation of the potent procoagulant factors tissue factor and plasminogen activator inhibitor-1 was inhibited by KLF2 overexpression. As a consequence of this differential effect on thrombotic gene expression, blood clotting time under flow condition was increased by KLF2. Conversely, small interfering RNA (siRNA)-mediated knockdown of KLF2 resulted in the opposite effects.58 This work identifies KLF2 as a key regulator of endothelial coagulant gene expression and function. Subsequent studies performed by Horrevoets and colleagues and by our group using genomic profiling approaches have also identified multiple additional factors in the thrombotic pathway regulated by KLF2 expression.51,52

Regulates Endothelial Proliferation, Migration, and Angiogenesis

In response to certain stimuli (eg, vascular endothelial growth factor [VEGF]), the normally quiescent endothelial cells can be induced to proliferate, migrate, and form new blood vessels. Angiogenesis is an important feature of both normal physiologic (eg, menstrual cycle) and pathologic states (eg, chronic inflammation and cancer).59–61 Studies from our laboratory and others demonstrate that KLF2 has potent antiangiogenic effects. Using the nude ear mouse model, we demonstrated that KLF2 overexpression inhibits VEGF-mediated angiogenesis and tissue edema. In addition, forced
overexpression of KLF2 inhibited the ability of VEGF to activate endothelial cells, as evidenced by a reduction in intracellular calcium influx, cell proliferation, and VEGF-induced proinflammatory factor expression (eg, VCAM-1, tissue factor, and cyclooxygenase 2). The molecular mechanism of this effect was shown to be at least partly attributable to the ability of KLF2 to inhibit the expression of the key VEGF receptor, VEGFR2/KDR, by competing with SP1 for binding to the VEGFR2 promoter. Studies by Horrevoets and colleagues, using standard wounding assays of HUVECs growing on a fibronectin matrix, observed that wounded monolayers overexpressing KLF2 exhibited greatly reduced migration. They also found that KLF2 overexpressing cells have decreased levels of VEGFR2. In addition, using gene profiling experiments, semaphorin-3F, a factor previously demonstrated to strongly inhibit tumor cell migration, was potently induced by KLF2. Thus by reducing endothelial cell proliferation and migration, KLF2 can inhibit angiogenesis.

Regulates Expression of Factors Implicated in Regulating Vasoreactivity and Vascular Tone

The normal healthy endothelium regulates vessel tone through the elaboration of several vasodilatory factors such as C-natriuretic peptide and eNOS. Under physiologic conditions, eNOS and C-natriuretic peptide are both induced by laminar blood flow. In pathologic conditions, the balance of vasodilatory and vasoconstrictive mediators shifts toward predominantly vasoconstrictive factors such as endothelin-1, a strong endogenous vasoconstrictor. Studies by SenBanerjee et al were the first to identify KLF2 as a potent inducer of eNOS expression and activity. KLF2 promoter deletion and mutational analysis revealed a single KLF2 site to be critical for the ability of KLF2 to bind and activate the eNOS promoter. Furthermore, this activation function was mediated by KLF2 and its recruitment of the coactivator CBP/p300 to the eNOS promoter (Figure 2). Subsequently, multiple studies have confirmed the ability of KLF2 to induce eNOS. In addition, these studies demonstrate that KLF2 can inhibit the expression of endothelin, adrenomedullin, and angiotensin-converting enzyme, 3 important genes in regulating vascular tone. Genomic profiling studies identified the ability of KLF2 to induce C-natriuretic peptide and arginosuccinate synthase, a limiting enzyme in eNOS substrate bioavailability. In addition, KLF2 decreased the expression of caveolin-1, a cell membrane protein that serves as a major negative regulator of eNOS activity. Loss-of-function studies using siRNA approaches have offered important validation of the effects of KLF2. Indeed, knockdown of KLF2 leads to reduced expression of eNOS and C-natriuretic peptide under basal and flow conditions.

Mechanisms Regulating KLF2 Expression in Endothelial Cells

Flow-Mediated Induction

As discussed in the introduction, laminar blood flow is thought to confer favorable properties to the endothelium. The absence of laminar flow is thought to contribute to atherogenesis in regions of the vascular tree that are exposed to nonlaminar blood flow such as branch points. This concept is not new, having been hypothesized by several great pathologists, including Virchow, Rokitansky, and Anischtkow. However, the exact mechanism of laminar shear-stress-mediated atheroprotection has remained poorly understood.

In 2002, using a gene profiling approach, Horrevoets and colleagues first demonstrated that KLF2 is induced by flow. Furthermore, these investigators showed that KLF2 is differentially expressed in the human vasculature. KLF2 is more highly expressed in the linear segments of the vessel and is decreased at branch points, which are the more atheroprone regions of the vasculature. These observations, coupled with the understanding that KLF2 can regulate the expression of many factors in a manner similar to flow, raised the possibility that this transcription factor may mediate some of the favorable effects of blood flow. The importance of KLF2 in regulating flow-mediated effects in endothelial cells was shown by Dekker et al, who demonstrated that knockdown of KLF2 prevented flow-mediated induction of eNOS and flow-mediated reduction of endothelin-1. Subsequent work by Parmar et al revealed that more than 15% of flow-regulated genes are dependent on flow-mediated KLF2 induction. In addition, of the most highly flow regulated genes, nearly 50% are dependent on KLF2 upregulation.

As a first step toward elucidating the mechanism of flow-mediated induction of KLF2, several laboratories have undertaken standard promoter analyses. The critical breakthrough in understanding the regulation of KLF2 expression occurred from studies by Kumar and colleagues, who identified a single consensus myocyte enhancing factor 2 (MEF2)-binding site in the conserved region of the KLF2 promoter (Figure 2). MEF2 factors are members of the MADS box (MCM1, Agamous, Deficiens, Serum response factor) family of transcription factors that bind to A/T-rich sequences. Although best known for their role in muscle development, an emerging body literature implicates MEF2A and MEF2C as critical regulators of endothelial biology. For example, mutations in MEF2A have been identified in an inherited disorder with features of coronary artery disease. Furthermore, MEF2C has been implicated as a regulator of endothelial integrity and permeability. Interestingly, MEF2-null mice have a similar phenotype to KLF2-null mice and die in the late embryonic stage. The exact basis of the favorable effects of MEF2 factors in endothelial cells remains unknown, but the link to KLF2 provides a potential explanation.

The identification of MEF factors as regulators of KLF2 expression also provided an immediate link to flow. Previous studies by Berk and colleagues identified extracellular signal-regulated kinase 5 (ERK5) (also known as Big mitogen-activated protein kinase 1 [BMK1]) as a highly flow-induced factor. One of the best characterized targets of ERK5 is the MEF2 family of transcription factors. The connection between KLF2 and this pathway was confirmed by 2 lines of evidence. Loss-of-function studies performed by Winoto and colleagues showed that ERK5 is essential for embryonic...
KLF2 expression and that ERK5 drives KLF2 transcription by activating MEF2 transcription factors. Consistent with this observation, Parmar et al showed that overexpression of a dominant negative MEF2 or mutant MEK5 (an upstream activator of ERK5) prevented flow-mediated induction of KLF2 expression in endothelial cells. In addition to MEF2, additional factors and mechanisms have been implicated in the flow-mediated induction of KLF2 expression. Using DNA affinity chromatography and mass spectrometry, Huddleson and colleagues identified p300/CBP-associated factor (PCAF), heterogeneous nuclear ribonucleoprotein D, and nucleolin as proteins that bind to the KLF2 promoter (Figure 2). Chromatin immunoprecipitation and gel-shift analysis demonstrate that these proteins bind the proximal KLF2 promoter as part of a phosphoinositide-3 kinase (PI3K)-dependent shear stress regulatory complex. Finally, recent studies also suggest that prolonged flow stabilizes KLF2 mRNA via a PI3K-dependent signaling pathway (Figure 2).

Cytokine-Mediated Inhibition
An important observation is that many proinflammatory stimuli inhibit KLF2 expression in endothelial cells.

Intuitively this makes sense. By reducing expression of antiinflammatory factors, noxious stimuli may therefore exact their proinflammatory effects in an unimpeded manner. The molecular basis for this inhibition of KLF2 expression has been recently elucidated. Using genetic and chemical inhibitors, Kumar and colleagues determined that both the NF-κB and histone deacetylase pathways were necessary for TNFα-mediated reduction of KLF2. A constitutively active form of the NF-κB inhibitor, IκB, completely abolished the inhibition of KLF2 expression by TNFα. In addition, TNFα was no longer able to inhibit KLF2 in p65-null cells. Lastly, treatment of cells with the histone deacetylase inhibitor trichostatin A completely abrogated the ability of TNFα to inhibit KLF2 expression.

A combination of promoter deletion and mutational analysis, communoprecipitation and chromatin immunoprecipitation assays, and siRNA “knockdown” experiments revealed that histone deacetylase-4/5 and p65 (a component of NF-κB) can form a trimolecular complex with MEF2 factors on the KLF2 promoter (Figure 2) and can inhibit the ability of MEF2 to induce KLF2 expression. This work suggests that endothelial activation by inflammatory cytokines is potentially mediated by reduction in KLF2 activity, leading to unopposed NF-κB activity and its subsequent deleterious effects.

KLF2 As a Mediator of Statin Effects
As discussed above, KLF2 confers favorable properties to the vascular endothelium, making this protein an ideal target for the therapeutic treatment of cardiovascular disease. Recently, there has been a large amount of investigation conducted on the 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (or statins). Multiple lines of evidence indicate that this family of drugs exhibits atheroprotective effects greater than those anticipated from LDL-lowering alone. Review of the literature reveals a large amount of overlap between the beneficial cellular effects of statins and those of KLF2. In this regard, studies from our group and others have identified a novel link between KLF2 and statins.

We and others have demonstrated that multiple statins can induce KLF2 expression (Figure 2). The Rho pathway is implicated, as statin-mediated KLF2 induction was abrogated by geranygeranyl pyrophosphate but not farnesyl pyrophosphate. In addition, constitutive Rho activity inhibited KLF2 induction. Promoter deletion and mutational analysis identified the MEF2-binding site of the KLF2 promoter as necessary for statin-mediated KLF2 induction. Finally, KLF2-knockdown experiments using siRNA demonstrated that KLF2 is required for statin-mediated induction of eNOS and TM mRNA and protein levels. These data strongly implicate KLF2 as a novel nuclear mediator of statin effects in endothelial cells.

Krüppel-Like Factor 4
KLF4 (also known as gut-enriched Krüppel-like factor [GKLF] or epithelial zinc-finger protein [EZF]) was originally identified as a KLF expressed in gut and skin epithelium. KLF4 is highly expressed in the differentiating layers of the epidermis, and systemic loss of this factor results in neonatal death attributable to loss of skin-barrier function. Furthermore, conditional deletion of KLF4 in gastrointestinal epithelium reveals an increased proliferation and altered differentiation of gastric epithelia, suggesting KLF4 is critical for normal gastric epithelial homeostasis. More recently, conditional deletion of KLF4 in the surface ectoderm-derived structures of the eye, where KLF4 is highly expressed, resulted in corneal epithelial fragility, stromal edema, and loss of conjunctival goblet cells. KLF4 has been extensively studied in cancer biology where it has been shown to be a cell growth inhibitor. Finally, KLF4 has recently been independently implicated by 2 independent groups as being important in maintaining pluripotent state of stem cells.

A potential role for KLF4 in endothelial biology was first provided by Yet and colleagues, who cloned this factor from an endothelial cell library. Subsequent efforts have shown that like KLF2, KLF4 expression is also flow inducible. However, the precise role of KLF4 in endothelial cell function has yet to be elucidated. Recent studies by Hamik et al implicate KLF4 as a regulator of endothelial activation in response to proinflammatory stimuli. These studies confirm, both in vitro and in vivo, that KLF4 is expressed in endothelial cells and demonstrate that KLF4 is induced by proinflammatory stimuli and shear stress. KLF4 overexpression induces several antiinflammatory and antithrombotic factors such as eNOS and TM, whereas knockdown of KLF4 enhances TNFα-induced VCAM-1 and tissue factor expression. Furthermore the functional significance of KLF4 is demonstrated by the observation that overexpression of this factor in endothelial cells markedly decreases inflammatory cell adhesion to the endothelial surface and prolongs clotting time under inflammatory states. Taken together, these observations support an important role for KLF4 in endothelial cell biology.
**Krüppel-Like Factor 6**

KLF6 (also known as core promoter element binding protein [COPPEB], GC-rich sites binding factor [GBF], or Zf9) was independently cloned from liver, placenta, and leukocyte cDNA libraries by a number of groups. 106–109 KLF6 was initially shown to be rapidly induced in activated hepatic stellate cells, the key fibrogenic cell type in liver injury and repair, implicating this factor as playing a role in tissue injury. 106,107 In addition, genes directly involved in the injury response, including collagen αI, TGF-β1, and types I and II TGF-β receptors are transactivated by KLF6 in hepatic stellate cells.107,110

In endothelial cells KLF6 has been shown to transcriptionally activate urokinase plasminogen activator, subsequently leading to the activation of latent TGF-β. 28 Urokinase plasminogen activator is a key enzyme implicated in tissue remodeling, tumor metastasis, and apoptosis. 111 In response to vascular injury, KLF6 interacts with Sp1 and cooperatively binds and transactivates the endoglin promoter.27 Endoglin is an endothelial membrane glycoprotein involved in vascular remodeling and cardiovascular development that is upregulated in response to arterial injury. More recently, KLF6 has been demonstrated to play a role in endothelial cell motility. 112 Farnesoid X Receptor activation leads to the disruption of an SP2/KLF6 repression complex on the matrix metalloproteinase-9 promoter via SHP (small heterodimeric partner). The resulting upregulation of matrix metalloproteinase-9 induces endothelial cell migration, a fundamental step in the process of vascular remodeling and repair. 112 Taken together, these results strongly implicate KLF6 as playing a key role in vascular development, remodeling and response to injury.

**Summary and Future Directions**

The studies discussed above support an important role for KLFs in endothelial biology. The most highly studied KLF in endothelial cells to date has been KLF2. The ability to differentially affect the expression of factors that confer anti-inflammatory, antithrombotic, and antiproliferative effects in endothelial cells provides the basis for the current zeitgeist that KLF2 may serve as a “molecular switch” regulating endothelial function in health and disease. Furthermore, the fact that a significant number of flow-inducible genes are KLF2-dependent suggests that this transcriptional mediator may also serve as a nuclear effector of the favorable effects of flow on endothelial gene expression and function. Despite the progress to date, many questions remain unanswered.

Although many of the functions of these endothelial transcription factors have been determined, the detailed molecular mechanisms have yet to be completely elucidated. What other factors may cooperate with KLFs to mediate their cellular functions in endothelial cells? Although basic structure/function analysis has been undertaken (Figure 1), detailed analysis of these factors has yet to be performed. Furthermore, many transcription factors are posttranslationally modified as a means of regulating their function. Very little is known about the posttranslational modification of endothelial KLFs and its role in mediating their functions.

The mechanotransduction complex that integrates flow signals into intracellular signaling pathways is currently being explored. 113–115 The MEK5/ERK5/MEF2 and PI3K pathways have been implicated as playing roles in the flow-mediated induction of KLF2. Are there other pathways or factors that are responsible for flow-mediated induction of KLF2? Finally, although KLF2 appears to be critical in flow-mediated regulation of endothelial cell function, it does not account for the full effects of flow. 51 Whether other flow-inducible KLFs, such as KLF4, also contribute to regulating endothelial cell gene expression remains poorly understood and is the subject of current investigation. As outlined in this review, initial studies indicate that KLF4 and KLF2 may have overlapping functions. Although confirmatory studies in vivo are clearly needed, these observations do suggest that this pair of KLFs function to maintain endogenous levels of key factors that regulate endothelial phenotype under both basal and inflammatory conditions.

The majority of the work on endothelial KLFs has been performed using in vitro studies. In the future, it will be of critical importance to further develop and study animal models, such as transgenic and conditional knockout mice, to confirm the in vitro results in an in vivo system. For example, although recent in vivo studies have outlined the importance of KLF2 in development, 40 the in vivo role of KLF2 in the adult vasculature under physiologic and pathologic states remains unknown. Given the known antiangiogenic effects of KLF2, one might speculate that transgenic mice overexpressing the gene may be more resistant to tumor formation, a process known to be heavily dependent on neovascularization. The field of KLFs in endothelial biology has made significant progress, but much work remains to be done. The answer to these questions and others could help identify novel therapeutic targets for improving endothelial function and treating or preventing vascular disease.

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**Disclosures**

None.

**References**


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