Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2014 March ; 34(3): 499–508. doi:10.1161/ATVBAHA.113.301925.

Regulation of an Inflammatory Disease: Krüppel-like Factors and Atherosclerosis

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Abstract

This invited review summarizes work presented in the Russell Ross lecture delivered at the 2012 proceedings of the American Heart Association. We begin with a brief overview of the structural, cellular, and molecular biology of Krüppel-like factors. We then focus on discoveries over the past decade implicating Krüppel-like factors as key determinants of vascular cell function in atherosclerotic vascular disease.

Keywords

Krüppel-like factor; atherosclerosis

Introduction to Krüppel-like factors

Krüppel-like factors (KLFs) are thusly named due to the amino acid sequence homology between their zinc finger (ZnF) domains and that found in the *Drosophila* transcriptional regulator *Krüppel* (*Kr*). In mammals, the KLF/SP family of transcription factors is characterized by 3 consecutive Cys_2/His_2 ZnF moieties located at their C-terminus and connected by a highly conserved seven residue interfinger sequence, TGEKP(Y/F)X [1–3] (Figure 1, panel A). To date 17 KLF genes and 9 Specificity Protein (SP) genes have been identified (with an 18th KLF recently predicted [4]) forming a family of ZnF-containing transcription factors that bind to GC-, GT- and CACCC-box motifs found in gene promoters and other regulatory elements [5]. The most distinguishing features that differentiate the KLF subfamily from SPs is the absence of both the SP box (located close to the N-terminus) and the SP hallmark, the Buttonhead (BTD) box (positioned just N-terminal to the ZnF domain). Using sequence analysis of the conserved 81-amino acid ZnF domain the KLF/SP family has been classified into subgroups of highly related genes; for informative cladograms and

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The authors report no conflict of interest.

evolutionary trees see [4–6]. Of import, homology between KLF family members is largely restricted to the DNA-binding zinc-finger region. They are highly divergent in their non-DNA binding regions — the domains that regulate transcriptional activation or repression — and it is proposed that the differing and sometimes opposing functions of different KLFs is a consequence of distinctive protein-protein interactions at these modulatory domains.

KLF expression is differentially regulated by physiologic stimuli, during cell differentiation, and in response to inflammatory cytokines. This results in a restricted expression pattern for some KLFs but a wide tissue distribution for many. KLFs have been implicated in diverse cellular processes including growth and differentiation, metabolism, and homeostasis. KLFs are largely found in the cell nucleus; however cytoplasmic localization of KLFs has been documented in response to stimulation with cytokines or calcineurin inhibitors, post-translational modification, or isotypic variation and depending upon the cell type cytoplasmic localization of KLFs has been shown to effect maturation, quiescence, phenotypic switching, or oncogenic potential [7–11]. Interestingly, accumulating evidence suggests that altered cellular function due to cytoplasmic localization of KLFs is not solely a consequence of the lack of access to nuclear contents, but that interaction of KLFs with cytoplasmic proteins can alter the function or stability of those proteins [12, 13].

Similar to *Kr* in *Drosophila*, the KLFs can act as either transcriptional activators or repressors, and exert their effects via either direct DNA-binding or through interaction with cofactors. There is accumulating evidence that context-dependent interactions are at the crux of the multifarious effects of KLFs. For example, in breast cancer cells KLF4 inhibits the expression of p53 (thus promoting cell proliferation) and also induces the cell-cycle inhibitor $p21^{CIP1/WAF1}$. In the context of the loss of $p21^{CIP1/WAF1}$ KLF4 is converted from a cell-cycle inhibitor to an oncoprotein [14]. In endothelial cells (ECs) and monocytes, we have demonstrated that the “context” alters the result of KLF interaction with transcriptional cofactors [15, 16]. Under basal (healthy) conditions in ECs, KLF2 and KLF4 interact with the cofactor p300 to augment transcription of eNOS and thrombomodulin. However, under inflammatory conditions, NF-κB translocates to the nucleus and interacts with p300 for maximal induction of target genes. By binding to p300 KLF2 or KLF4 can sequester this cofactor from NF-κB and thus attenuate NF-κB-dependent inflammatory gene expression [16]. Thus, KLF interaction with p300 can result in either transactivation or repression, depending upon context (Figure 1, Panel B). Indeed, transcriptional regulation via interaction with p300 is thematic for several KLFs [15, 17–21].

Atherogenesis

Manifesting clinically as myocardial infarction, stroke and peripheral vascular disease with an enormous impact on health, atherosclerosis has garnered the greatest attention amongst the various pathologies afflicting vessels. The anatomic calling card of this disease is the co-optation of vessel walls by a fatty, inflammatory, calcific agglomeration. Cumulative clinical and experimental observations led Ross and Glomset to propose the “response to injury” hypothesis, published as a two-part series in the New England Journal of Medicine [22, 23]. In its original incarnation, the theory proposed that endothelial desquamation allowed platelets to adhere to the subintima and thus initiate the disease. Repetition of this injury would lead

to investment of additional cells (e.g. leukocytes) and progression of disease. While the hypothesis would undergo significant modification over the years, with increasing importance given to the centrality of endothelial dysfunction and importance of inflammation in disease pathogenesis, the model was critical in providing an envisaging framework for investigators in contemplation of this complex disease [24].

The convergence of cell and molecular tools over the past several decades have facilitated major advances in our understanding of nodal pathways operative within vessel-intrinsic and -extrinsic cells that control the development of atherosclerosis. In this review we focus on the discovery of a key role in atherogenesis for KLFs. For simplicity, we will consider atherogenesis as a multi-phase process that converts a healthy artery to one occluded by atherothrombosis by focusing on dysfunction of a particular cell type that distinguishes transition between each phase of this chronic disease (Figure 2). The consequences of altered KLF function at each phase will be discussed, followed by an outline of the current understanding of the mechanistic details.

Healthy artery → Endothelial Dysfunction

The healthy artery is lined by a confluent endothelial monolayer that elaborates substances that create a non-adhesive, non-thrombotic barrier between the flowing blood and underlying tissue. Interendothelial junctions tightly regulate permeability of fluid and macromolecules across the endothelial surface. Dynamic signaling between blood-borne humoral factors, metabolic products of underlying tissues, and chemical communication between the endothelium and the vascular smooth muscle cells of the medial layer of the vessel control vasomotor tone. Central to maintenance of this homeostasis is optimal endothelial function, and laminar shear stress (LSS) created by blood flowing over the endothelium is a highly potent effector in this respect. Evidence from our group and others demonstrates that members of the KLF family, particularly KLF2 and KLF4, are central players in this shear stress-mediated homeostasis.

KLF2 and KLF4 are highly expressed in ECs exposed to laminar flow and reduced in ECs at arterial branch points and other regions of turbulent flow, such as the inferior aspect of the aortic arch [16, 25–29]. Anatomically, this correlates to regions long recognized as most prone to development of atherosclerotic lesions. In the last five years, in vivo animal experiments have demonstrated that EC deficiency of KLF2 and KLF4 also predispose to atherosclerosis. In mice deficient in apolipoprotein E and thus susceptible to diet-induced atherosclerosis (the “ApoE model”), KLF2 hemizygous mice have increased plaque burden [30]. EC-specific loss of KLF4 also increases the severity of atherosclerotic lesion formation whereas EC-specific overexpression of KLF4 is protective [16]. In vitro and in vivo data demonstrate that both KLF2 and KLF4 are potent inducers of eNOS and thrombomodulin expression and also inhibit cytokine-induced expression of adhesion factors-including VCAM-1, E-selectin- and other inflammatory mediators [26, 27]. Recent studies demonstrate that KLF2 plays an essential role in maintaining endothelial barrier integrity, including protection from ischemic stroke, by differential regulation of key junction proteins (e.g. ZO-1 and occludin) [31, 32]. In vivo permeability studies on KLF4 are limited thus far; however, KLF4 is likely to influence permeability via transcriptional regulation of VE-cadherin [33].

Determination of the precise mechanisms by which LSS induces KLF2 and KLF4 expression remains an active area of investigation for numerous laboratories. Primary EC cilia are postulated to be a shear stress sensor in areas of low or disturbed flow [34]. ECs from mice with genetic loss of cilia fail to induce KLF2 and downregulate KLF4 in response to LSS. These non-ciliated cells do not express eNOS in response to shear; instead, they lose their cobblestone appearance and acquire a mesenchymal-transitional phenotype [35, 36]. While fascinating, mechanotransduction via cilia may be most important during cardiovascular development or limited to areas of low shear stress as EC cilia are not found, nor required for response, in areas with high shear stress. Indeed, constitutive, endothelial-specific KLF2 null mice die at embryonic day 10.5 secondary to lack of vascular tone, bleeding and cardiac dysfunction [37, 38].

A molecular link between flow and its downstream effects is signaled, in part, by MAPK (mitogen-activated protein kinase) pathways that activate ERK. KLF2 and KLF4 are induced by ERK activation. The KLF2 promoter is upregulated by MEF2 binding downstream to both the MEK5/ERK5/MEF2 and the AMPK/ERK5/MEF2 flow pathways [39, 40]. ERK5-dependent KLF4 induction confers the vasoprotective phenotype described by enhanced expression of anti-thrombotic, hemostatic, and vasodilatory genes [41]. Post-transcriptional regulation also appears to play a role both in endogenous regulation of KLF2 and KLF4; two laboratories independently found that inhibition of miR-92a by atheroprotective flow allows for increased levels of EC KLF2 and KLF4 [28, 42].

Other KLFs

KLF6 expression is induced in EC after vascular injury and, in cooperation with Sp1, induces several target genes involved in vascular remodeling, including endoglin, collagen α1, TGFβ1 [43, 44], and activin receptor-like kinase-1 [45]. In in vitro scratch assays, overexpression of KLF6 leads to more rapid “wound healing” and enhanced EC migration [45, 46]. KLF11 suppresses NF-κB-mediated EC activation, a role that may be particularly important in diabetes mellitus [47]. Unproven as yet is the ability of KLF6 or KLF11 to alter atherogenesis, although it has been shown that KLF6 levels are upregulated in primary human monocytes after knockdown of the potent anti-inflammatory macrophage transcriptional regulator tristetraprolin [48]. In regard to KLF2 and KLF4, however, the data is compelling for a central role in maintaining EC homeostasis and vascular health.

Endothelial Dysfunction → Immune Cell Infiltration

As a consequence of EC activation blood leukocytes adhere to the luminal surface of the vessel and transmigrate into the intima via dysregulated interendothelial junctions [49]. Therein monocytes differentiate to macrophages, which then take up oxidized low-density lipoprotein (OxLDL) and become foam cells, an essential characteristic of the atherosclerotic lesion, manifesting early in disease as a “fatty streak” [50]. Lipid-laden macrophages in turn further activate ECs, enhancing secretion of chemokines and expression of cell adhesion molecules, directing more monocytes and T-cells to move into the vessel wall, and thus leading to local activation of both innate and adaptive immunity and disease progression [49–51]. Macrophages participate in atherogenesis by being central cellular

effectors of both thrombotic and inflammatory signals [52]. Arrested development of atherosclerosis is evident after depletion of monocyte/macrophages or manipulation of cytokine-mediated recruitment of these cells. [53–55]. Several members of the KLF family, in particular KLF2 and 4, have emerged as important regulators of monocyte/macrophage biology.

Myeloid Cells

As factories of both cytokine and protein mediators of inflammation macrophages are central to the development atherosclerosis [56]. There is strong data implicating KLFs in governance of myeloid activity during atherogenesis.

KLF2—KLF2 is a potent negative regulator of monocyte/macrophage proinflammatory activation and an essential regulator of the innate immune response [18, 21]. While there is important data elucidating the role of KLF2 in acute inflammation (sepsis), due to space limitations we will focus here on chronic inflammation apropos of atherosclerosis. Patients with coronary artery disease have significantly lower KLF2 expression in circulating monocytes than do healthy subjects [18, 21]. Animals with myeloid-specific deletion of KLF2 have elevated baseline plasma levels of proinflammatory molecules including as IL-1β and TNFα supporting the idea that KLF2 is a tonic repressor of myeloid activation [21]. LDLR null mice with myeloid-specific KLF2 deletion develop a markedly greater atherosclerotic burden than controls [57]. Mechanistically, there is data that supporting a role for myeloid KLF2 deficiency in enhancing macrophage adherence to ECs [57] and inducing OxLDL uptake [30], thus augmenting macrophage-derived foam cell formation.

KLF4—Gain- and loss-of-function studies show that KLF4, as a downstream target of PU.1, promotes monocyte differentiation [58]. In vivo experiments in KLF4 −/− chimeric mice demonstrate a role of KLF4 in inflammatory monocyte differentiation, with KLF4 expression necessary for maturation of both $Ly6C^{hi}$ and $Ly6C^{lo}$ monocyte populations [59]. Beyond developmental biology, KLF4 is also a mediator of macrophage subset specification by regulating macrophage M1/M2 polarization [15]. In response to M2 stimuli (IL-4, IL-13) KLF4 expression is induced and expression remains high in the anti-inflammatory M2 macrophages. Conversely, KLF4 expression is suppressed by M1 polarization stimuli (LPS, IFNγ) and KLF4 levels in proinflammatory M1 macrophages are low. In line with these results, KLF4-deficient macrophages have increased proinflammatory gene expression and enhanced bactericidal effects. Pathophysiological effects including delayed wound healing, increased insulin resistance, and diet-induced obesity have been observed in animals with myeloid-specific deletion of KLF4. Mechanistically, KLF4 promotes the M2 phenotype by cooperating with STAT6 to promote M2 targets. KLF4 inhibits the M1 phenotype via inactivation of NF-κB by sequestration of the critical coactivators p300 and PCAF, as described above in ECs. KLF4 expression is downregulated in circulating monocytes of patients with coronary artery disease [60]. A recent study from our group confirms that myeloid KLF4 has an atheroprotective effect. Myeloid KLF4-deficient animals on an ApoE null background develop significantly more vascular inflammation and atherosclerotic lesion burden than control mice [61]. Collectively, these observations substantiated a major role of KLF4 in monopoiesis, macrophage polarization, and macrophage-driven pathology.

Other KLFs

Other KLFs including KLF1, KLF3 and KLF10 are expressed in myeloid cells and may participate in vascular inflammation and atherosclerosis [2, 62, 63].

T lymphocytes—Activation of naïve T cells leads to proliferation, enhancement of effector functions, and homing to areas of inflammation. This induction of adaptive immunity plays a role in progression of atherosclerosis [64]. Our group and others have demonstrated a role for KLF2, 4, 10 and 13 in regulation of adaptive immunity, with implications for atherosclerosis.

KLF2—Gain-and loss-of function approaches have demonstrated a role of KLF2 in maintaining T-lymphocyte quiescence associated with the ability of KLF2 to inhibit c-Myc expression and upregulate cyclin-dependent kinase inhibitor $p21^{CIP1/WAF1}$ [65, 66]. In addition, KLF2 serves a major role as a regulator of T- and B-cell survival and migration [67]. Thymocytes deficient in KLF2 have diminished expression of surface receptors and trafficking molecules such as the sphingosine-1-phosphate (S1P) receptor S1P1R, L-selectin, CCR7, and integrin β7. T cells lacking KLF2 express proinflammatory chemokine receptors such as CXCR3, CCR3, CCR5 and CD69 on memory cells and activated thymocytes [68]. These changes may lead to altered homing patterns of naïve T cells to nonlymphoid tissues and attenuation of T-cell proliferation. While not yet proven to effect atherosclerosis per se, an anti-inflammatory effect of KLF2 overexpression has been demonstrated via attenuation of a mouse model of T-cell dependent myocarditis [69].

KLF4—Differentiation of helper T cells type 17 (Th17) and the expression of IL-17 by these cells are regulated by KLF4 [70, 71]. While IL-17 has been shown to promote chronic inflammatory diseases such as arthritis, colitis, and autoimmune encephalitis, the role of the IL-17/Th17 in the development of atherosclerosis remains controversial. The presence of IL-17/Th17 has been demonstrated in both human and mouse atherosclerotic lesions, yet different mouse models have suggested either atheroprotective or proatherogenic roles [50, 72]. KLF4-deficient mice have a significant reduction in the severity of autoimmune encephalomyelitis attributed to attenuation of Th17 responses and infiltration of leukocytes into the central nervous system [71]. Taken together, these studies suggest a role of KLF4 in regulating T cell activation and proliferation, which may parlay into an effect on atherogenesis.

KLF10—Several studies have defined an atheroprotective role of regulatory T cells (Treg). KLF10 drives CD4+CD25− T cell activation and Treg differentiation and suppressor function [73]. Overexpression of KLF10 in Treg induces expression of TGFβ1, an atheroprotective cytokine. Importantly, in an ApoE null, scid background, adoptive transfer of KLF10 deficient CD4+CD25− T cells accelerates atherosclerosis.

KLF13—KLF13 was initially identified as a transcription factor expressed in activated T lymphocytes [74]. KLF13 enhances expression of the proinflammatory chemokine, RANTES, to promote T cell activation and attenuates promoter activity of a potent antiapoptotic factor, BCL-XL, to promote T cell and survival [75].

B lymphocytes—The role of B-cells in atherosclerosis is incompletely understood; however, B lymphocytes are found in the plaque and adventitia at areas of advanced atherosclerosis [64]. Evidence supporting a role of KLF3 in innate and humoral immunity includes effects on B cell differentiation and quiescence, as well as regulation of the proliferative response to LPS via attenuation of the toll-like receptor signaling pathway [76, 77]. KLF4 expression is found throughout all stages of B cell development and it has been demonstrated to take part in regulation of activation-induced B cell proliferation via induction of $p21^{CIP1/Waf1}$ expression and downregulation of c-Myc and cyclin D2 [78].

Immune Cell Infiltration → VSMC Proliferation

In a healthy artery, VSMC are located in the medial layer, separated from the ECs and intima by the internal elastic lamina. Their primary function is to respond to blood-borne, EC-derived, and tissue metabolic signals and relax or contract, controlling vasomotor tone. Synthetic activity and proliferative rate are very low, and they express markers of a contractile cellular phenotype, including SM a-actin, SM myosin heavy chain, h1-calponin, and smoothelin (reviewed in [79]). During atherogenesis, intimal infiltration of activated leukocytes creates a state of continued cellular crosstalk that amplifies the inflammatory response and results in chronic inflammation. In response to growth factors and cytokine (and perhaps additional undefined mechanisms) VSMC migrate across the elastic lamina into the subendothelial space, and transition of the lesion from a fatty streak to a more complex, bulky atherosclerotic plaque that may impair blood flow enough to cause angina. Mature VSMC retain remarkable plasticity, and it is assumed that they undergo a “phenotypic switch” from a relatively quiescent contractile cell, to an inflammatory, proliferative cell during atherogenesis, though details of the changed expression profile have been rigorously documented in vitro [79]. The relocated VSMC may proliferate (neointimal formation), take on characteristics of foam cells, elaborate inflammatory signals, and synthesize extracellular matrix proteins that lead to development of fibrous plaque cap [24, 80]. There is evidence for KLF4, 5, and 15 as effectors of the VSMC response in atherogenesis.

KLF4—KLF4 is not expressed in VSMC of the healthy artery; however, after vascular injury expression is rapidly induced and corresponds to loss of VSMC differentiation markers that characterize the contractile phenotype. In KLF4-deficient mice VSMC neointimal proliferation enhanced [81]. The Owens laboratory has demonstrated that OxLDL induces both phenotypic switching and enhanced VSMC migration in a KLF4-dependent fashion [82, 83]. Control of VSMC proliferation by KLF4 is mediated via induction of p53 and $p21^{CIP1/WAF1}$, reminiscent of mechanism in other cell types [81, 84]. KLF4 inhibits expression of VMSC differentiation genes by interfering with expression and function of the potent SMC coactivator myocardin [85]. Interestingly, KLF4 inhibits myocardin by binding to the promoter in cooperation with NF-κB [86]. Thus, in contrast to ECs, KLF4 is pro-inflammatory by cooperating with NF-κB in activated. Direct evidence, via VSMC-specific genetic gain- and loss-of-function experiments, that altered VSMC KLF4 alters atherogenesis is lacking as yet; however, recent studies utilizing miRNA approaches are supportive. MicroRNA-145 is highly expressed in arteries, but is attenuated after vascular injury and in atherosclerosis [87, 88]. Overexpression of miR-145 limits neointimal formation after vascular

injury, regulating the VSMC phenotypic switch between contractile and proliferative states, and it significantly reduces KLF4 levels (amongst other targets) [87, 88]. Of note, VSMC-specific lentiviral overexpression of miR-145 reduces KLF4 levels, limits atherosclerosis, and enhances plaque stability in the ApoE null mouse model[89].

KLF5—KLF5 expression in VSMCs is induced after vascular injury and in atherosclerotic lesions [90–92]. Genetic deficiency of KLF5 leads to baseline thinning of the medial and adventitial walls of arteries and inhibition of neointimal proliferation after vascular injury [93]. A potential pro-atherogenic role for KLF5 is also suggested by the gene profile it activates. Targets include PDGF-A, TGFβ1, cyclin B, Egr-1, and PAI-1; genes that enhance proliferation, migration and vascular inflammation [94, 95]. It will be of great interest to see the atherosclerotic phenotype of VSMC-specific KLF5 modulation.

KLF15—In contrast to KLF4 and KLF5, KLF15 is robustly expressed in VSMCs under basal conditions, but is attenuated after injury in mouse models and in human atherosclerotic tissue [96, 97]. In mouse models global deficiency of KLF15 leads to enhanced susceptibility to both heart failure and aortic aneurysm and, supportive of translation to human disease, KLF15 levels are reduced in human aortic aneurysms [98]. Indeed, VSMC-specific loss of KLF15 enhances atherosclerosis and vascular inflammation in the ApoE mouse model. True to our theme, KLF15 reduces activity of NF-κB on inflammatory gene promoters via direct interaction with p300 [97]. These observations provide the most stringent evidence to date implication a VSMC-intrinsic role in atherosclerosis for any KLF.

VSMC Proliferation → Atherothrombosis

Acute coronary syndrome results from plaque rupture with exposure of the blood to plaque lipids and tissue factor and thus initiation of the coagulation cascade, followed by platelet adherence and arterial thrombosis. Unfortunately, to date there are no reliable animal models that allow for quantitative assessment of plaque rupture and acute thrombosis, and thus data on the effect of KLFs on this aspect of atherosclerosis are limited. Indirect evidence does suggest however, that both EC KLF2 and KLF4 would be beneficial in protecting against atherothrombosis. KLF2 inhibits blood clotting in EC cultures [99]. Ex vivo (fibrin clot) and in vivo (carotid injury) experiments demonstrate that KLF4 protects against thrombosis, even in the presence of inflammation [16]. EC KLF2 and KLF4 increase thrombomodulin and tissue plasminogen activator expression (tPA) and decrease plasminogen activator inhibitor (PAI-1) and cytokine-stimulated tissue factor expression [27, 99], consistent with an anti-thrombotic effect. One can speculate that myeloid KLF2 and KLF4 might have a protective effect on plaque rupture-mediated thrombosis by inhibiting leukocyte expression of matrix metalloproteinases (MMPs) [21]. Finally, as mentioned above, miR-145-mediated downregulation of VSMC KLF4 may enhance stability of atherosclerotic plaque [89].

Conclusion

Atherosclerosis is a complex, chronic, highly morbid disease; the leading cause of death in the United States as well as increasing parts of the rest of the world. Cardiovascular physicians have slew of powerful medical therapies that have attenuated the mortality of the

disease, yet ~715,000 Americans suffer from myocardial infarction each year [100]. Krüppel-like factors have potent effects on a broad range of vascular processes that contribute to atherogenesis (summarized in Table 1) and the cumulative data is sufficient to warrant their consideration as therapeutic targets. While exercise, healthy diet, and a tobacco-free lifestyle would likely be the most effective way to prevent atherosclerosis, adoption of these lifestyle changes has not proven reliable. Thus, although the benefits of these modalities are potentially mediated by vascular KLF expression—exercise increases laminar shear stress and thus EC KLF2 and KLF4 [101], components of the "Mediterranean diet" including broccoli, grapes, red wine (resveratrol), and olive oil enhance KLF4 and KLF2 expression or reduce KLF6 [102–106], and cigarette smoke increases VSMC KLF4 expression [107] —real-life conditions may be insufficient to effect change in vascular KLF levels and thus drug discovery studies are ongoing to find more specific, potent regulators. HMG CoA reductase inhibitors (statins) are commonly used for treatment of coronary artery disease and induce EC expression of KLF2 and KLF4 [41, 69, 105, 108, 109]. Other creative modes of altering vascular KLF expression are also being explored; groups are assessing whether stents coated with agents that regulate KLF2 or KLF4 may improve neointimal hyperplasia or stent thrombosis [110–112]. Especially exciting is a recent study that used EC-derived extracellular vesicles to control gene expression in co-cultured VSMCs and reduce atherosclerotic lesion formation in ApoE null mice [113]. Hergenreider et al. exposed ECs to shear stress or lentiviral-mediated overexpression of KLF2. Extracellular vesicles secreted from these cells were enriched in the mir143/145 and were atheroprotective. This is very exciting news for fans of the KLFs, and of great interest for all those fascinated by communication between ECs and VSMCs. We are confident Dr. Ross would continue to find this topic utterly enthralling.

Acknowledgments

This work was performed with the support of NIH grants HL110630, HL097593, HL112486, HL086548, HL076754 (Mukesh K. Jain) and HL113570 (Anne Hamik).

Abbreviations

EC	endothelial cell
ET-1	endothelin-1
FABP4	fatty acid binding protein 4
IC	immune cell
ICAM-1	intercellular adhesion molecule-1
INFγ	interferonγ
KLF	Krüppel-like factor
Kr	*Krüppel*
LPS	lipopolysaccharide

LSS	laminar shear stress
MMPs	matrix metalloproteinases
NF-κB	nuclear factor κB
NO	nitric oxide
OxLDL	oxidized low-density lipoprotein
PAI-1	plasminogen activator inhibitor-1
PDGF	platelet-derived growth factor
SP	Specificity Protein
TF	tissue factor
TLR	toll-like receptor
TM	thrombomodulin
TNFα	tumor necrosis factorα
tPA	tissue plasminogen activator
VCAM-1	vascular cell adhesion molecule-1
VSMC	vascular smooth muscle cell
ZnF	zinc finger

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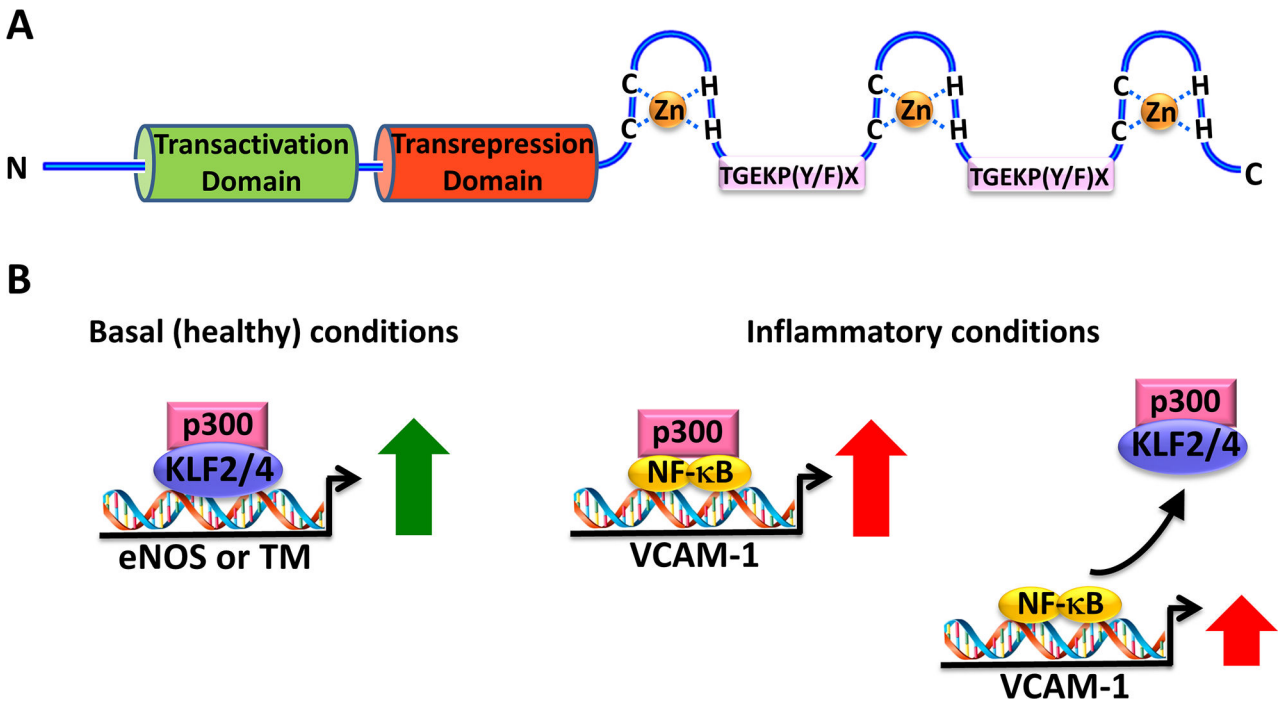
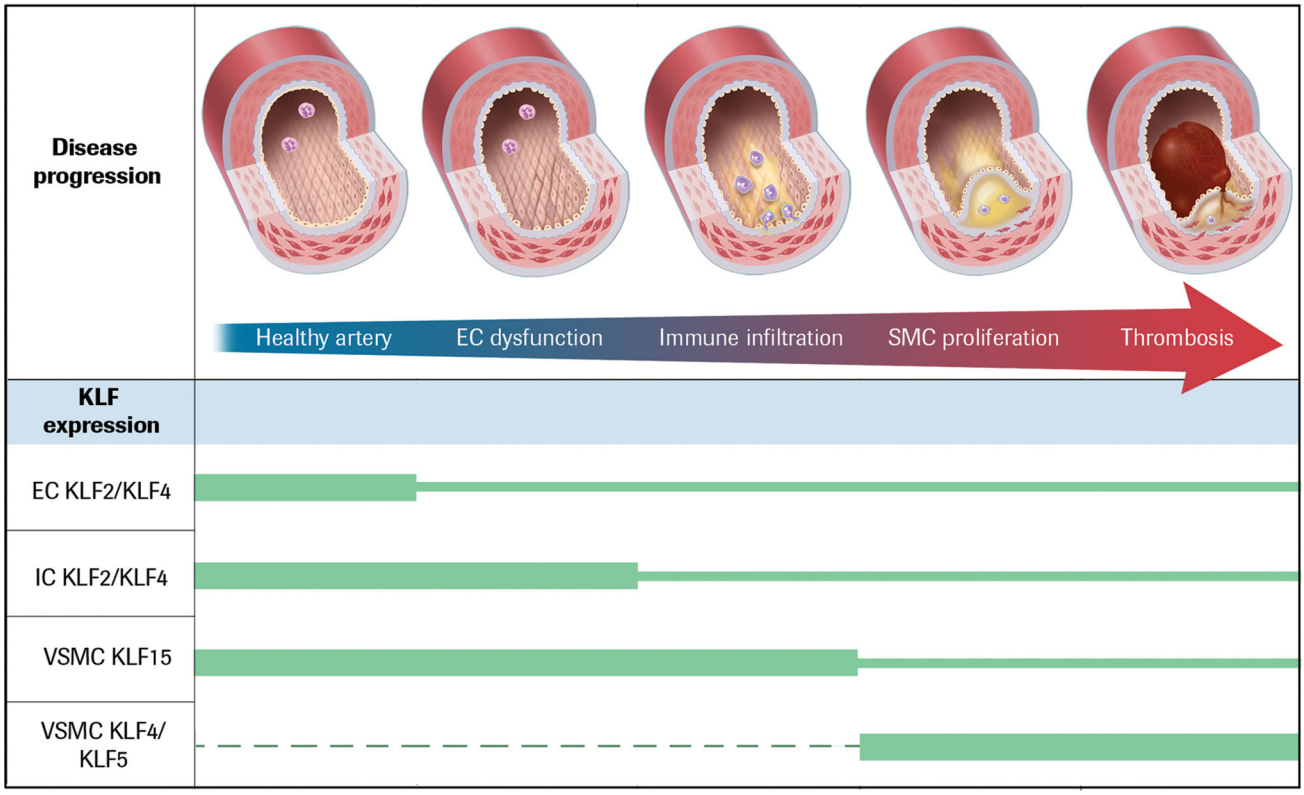
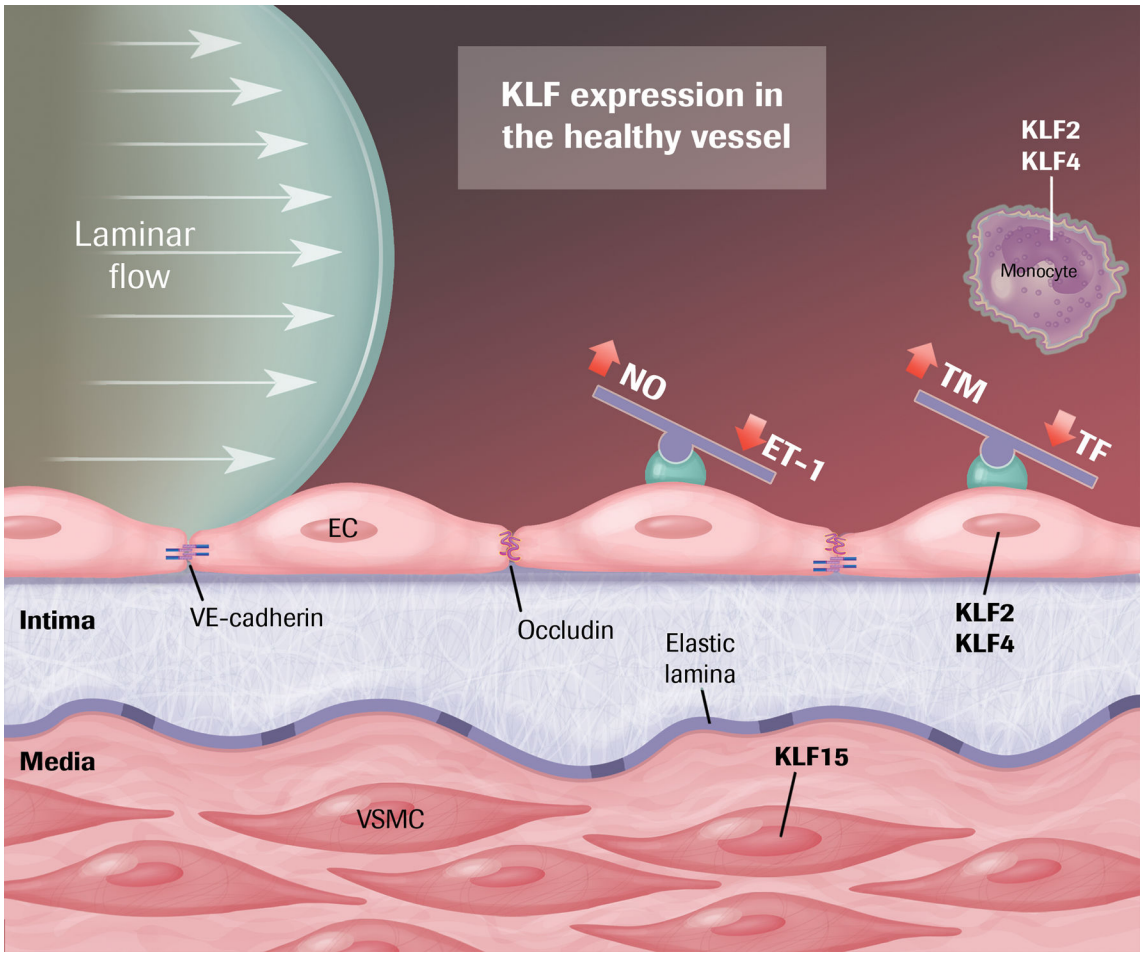
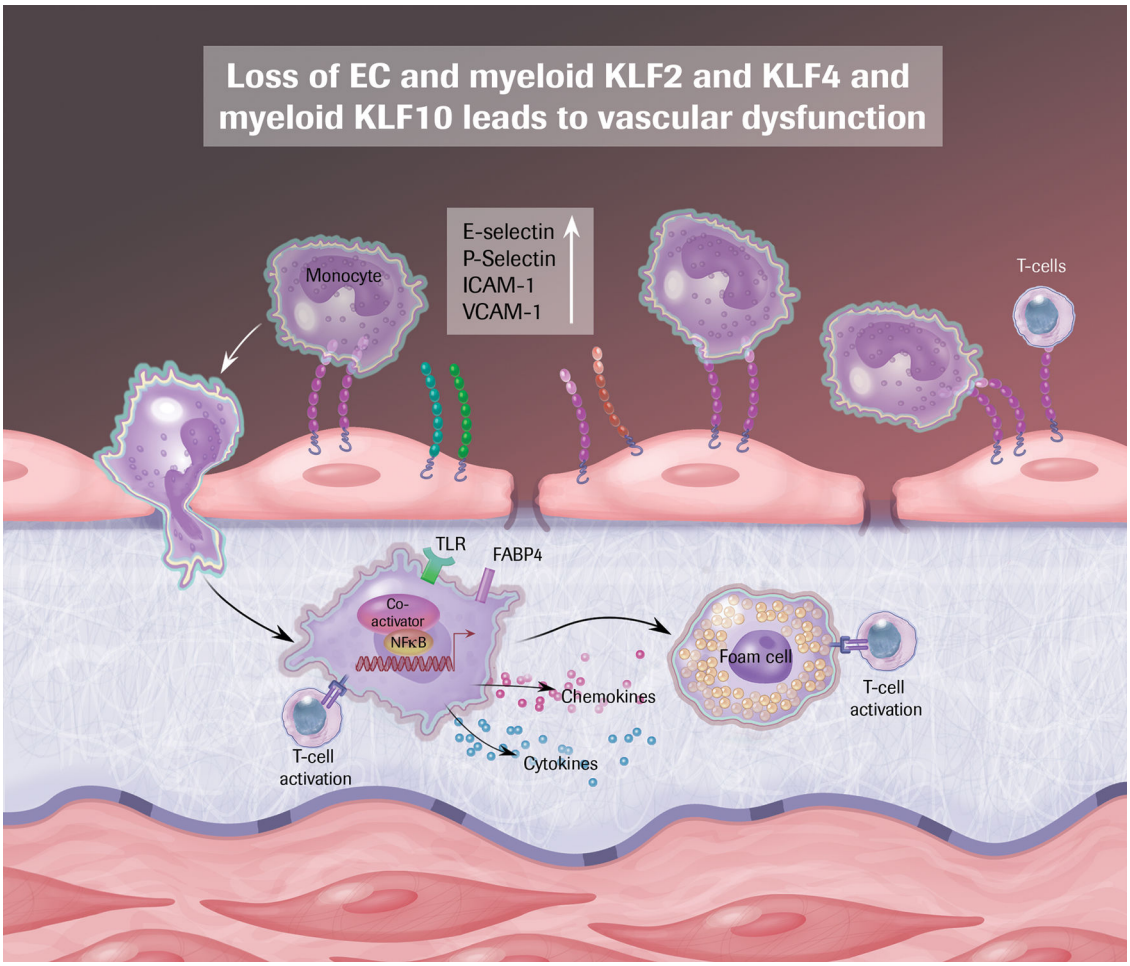


Figure 1.

(A) Schematic representation of common structure and functional domains for KLFs. The transactivation and transrepression domains are located at the N-terminal end. Three consecutive zinc finger moieties are located at the extreme C-terminus. A highly conserved 7-amino acid sequence, abbreviated TGEKP(Y/F)X, resides in the interfinger regions and contributes to DNA-binding. (B) Diagram illustrating one way the KLFs can have both transactivating and repressive functions. In this example, KLF2 or KLF4 interact with the cofactor p300 to augment transcription of eNOS and TM under basal conditions (left), or compete for binding to limiting amounts of the cofactor p300 to inhibit NF-kB-induced expression of proinflammatory genes (e.g. VCAM-1) (right).







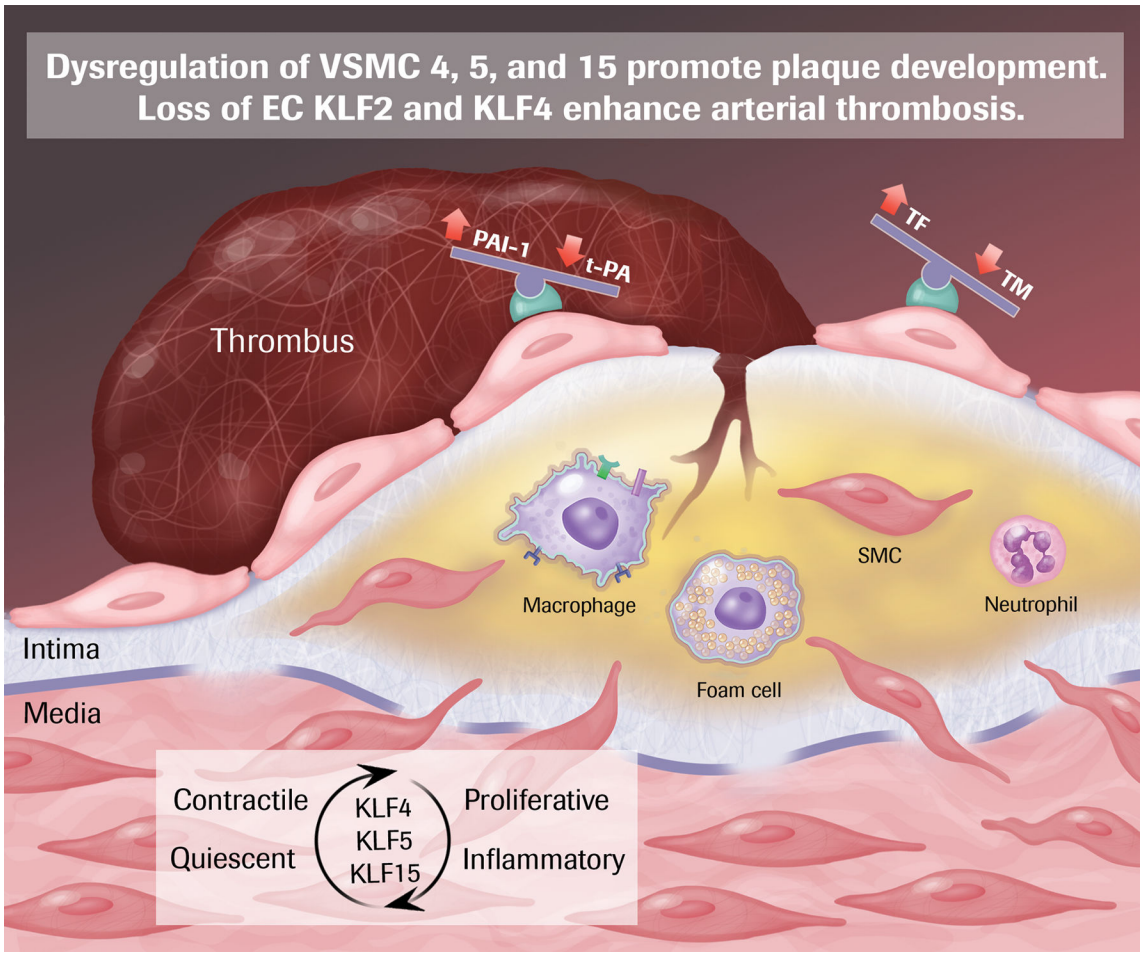


Figure 2.

Development of an atherosclerotic lesion, from healthy vessel to artery occluded by atherothrombosis. Relative levels of cell-specific expression of KLFs during the process of atherogenesis are indicated by the size of the green bars. The three pop-up figures provide details of the molecular changes occurring in the vascular cells and refer to the 1st (left-most), the 2nd and 3rd, and the 4th and 5th vessel cut-away images, respectively.

Table 1

Summary of expression and function of KLFs in regard to atherosclerotic vascular disease

See manuscript text for details, abbreviations, and references.

Cell type	KLF	Function
Endothelial cell	KLF2	• Promotes anti-inflammatory and anti-thrombotic phenotypes by inducing eNOS, TM and tPA and by inhibiting cytokine-induced expression of adhesion factors (e.g. VECAM-1 and E-selectin) and PAI-1. • Maintains endothelial barrier integrity by inducing expression of key junction proteins (e.g. ZO-1 and occludin).
	KLF4	• Promotes anti-inflammatory and anti-thrombotic phenotypes by inducing eNOS, TM and tPA, and by reducing expression of VCAM-1, E-selectin and PAI-1.
	KLF6	• Promotes vascular remodeling (in cooperation with Sp1) by inducing endoglin, collagen α1, TGFβ1, and activin receptor-like kinase-1.
	KLF11	• Attenuates NF-κB-mediated EC activation.
Immune cell -Myeloid Cell	KLF2	• Tonic repressor of myeloid activation by inhibiting the NF-κB pathway. • Inhibits macrophage-derived foam cell formation by regulating expression of macrophage lipid binding protein FABP4. • Inhibits leukocyte expression of MMPs, implying protection against plaque rupture-mediated thrombosis.
	KLF4	• Promotes an atheroprotective phenotype. • Promotes inflammatory monocyte differentiation (as a downstream target of PU1). • Regulates macrophage subset specification and macrophage polarization by cooperating with STAT6 to promote M2 targets and by sequestration of the critical coactivators p300 and PCAF to inhibit the M1 phenotype. • Inhibits leukocyte expression of MMPs, implying protection against plaque rupture-mediated thrombosis.
Immune cell -T lymphocyte	KLF2	• Maintains T lymphocyte quiescence by inhibiting c-Myc, and by inducing p21CIP1 expression. • Required for the expression of surface receptors and trafficking molecules such as L-selectin, CCR7, integrin β7 and the sphingosine-1-phosphate receptor S1P1R. • Promotes an anti-inflammatory phenotype in a mouse model of T-cell dependent myocarditis.
	KLF4	• Regulates differentiation of Th17 cells and IL-17 production by binding to IL-17 promoter.
	KLF10	• Promotes an atheroprotective effect by inducing expression of TGFβ1; by regulating CD4+CD25-T cell activation, T regulatory cell differentiation and Treg cell suppressor function.
	KLF13	• Promotes T cell activation by inducing expression of the proinflammatory chemokine, RANTES, and T cell survival by attenuating BCL-XL promoter activity.

Cell type	KLF	Function
Immune cell -B lymphocyte	KLF3	• Regulates B cell differentiation and quiescence, and B cell proliferative response to LPS via downregulation of toll-like receptor signaling pathway.
	KLF4	• Regulates activation-induced B cell proliferation by inducing p21CIP1 and downregulating c-Myc and cyclin D2.
Vascular smooth muscle cell	KLF4	• Level is rapidly induced in response to vascular injury. • Critical for VSMC phenotypic switching between contractile and proliferative states. • Enhances VSMCs migration in response to OxLDL. • Cooperates with NF-κB to inhibit expression of VSMC differentiation genes.
	KLF5	• Enhances VSMC proliferation, migration and vascular inflammation by inducing expression of proatherogenic genes (e.g. PDGF-A, TGFβ1, cyclin B, Egr-1, and PAI-1).
	KLF15	• Level is reduced after vascular injury in mouse models and in human atherosclerotic disease and aortic aneurysms. • Loss results in pro-atherosclerotic and pro-inflammatory phenotypes in mouse models. • Reduces activity of NF-κB on inflammatory gene promoters via direct interaction with p300.