Regulation of an Inflammatory Disease
Krüppel-Like Factors and Atherosclerosis

Mukesh K. Jain, Panjamaporn Sangwung, Anne Hamik

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 during the past decade, implicating Krüppel-like factors as key determinants of vascular cell function in atherosclerotic vascular disease. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: atherosclerosis  Krüppel-like transcription factors

Krüppel-like factors (KLFs) are thus named because of 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/specificity protein (SP) family of transcription factors is characterized by 3 consecutive Cys/His, ZnF moieties located at their C terminus and connected by a highly conserved 7-residue interfinger sequence, TGEKP(Y/F)X1–3 (Figure 1A). To date, 17 KLF genes and 9 SP genes have been identified (with an 18th KLF recently predicted) 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. The most distinguishing features that differentiate the KLF subfamily from SPs is the absence of their C terminus and connected by a highly conserved 7-residue interfinger sequence, TGEKP(Y/F)X1–3 (Figure 1A). 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 box (positioned just N-terminal to the ZnF domain). Using sequence analysis of the conserved 81-aa ZnF domain, the KLF/SP family has been classified into subgroups of highly related genes; for informative cladograms and evolutionary trees, see references 4 to 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 are a consequence of distinctive protein–protein interactions at these modulatory domains.

KLF expression is differentially regulated by physiological stimuli, during cell differentiation, and in response to inflammatory cytokines. This results in a restricted expression pattern of 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 on the cell type cytoplasmic localization of KLFs has been shown to effect maturation, quiescence, phenotypic switching, or oncogenic potential. Interestingly, accumulating evidence suggests that altered cellular function because of 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.

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

endothelial NO synthase and thrombomodulin. However, under inflammatory conditions, nuclear factor-xB (NF-xB) 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-xB and thus attenuate NF-xB–dependent inflammatory gene expression. Thus, KLF interaction with p300 can result in either transactivation...
or repression, depending on context (Figure 1B). 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 among 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 Glomset22,23 to propose the response to injury hypothesis, published as a 2-part series in the *New England Journal of Medicine*. 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 (eg, leukocytes) and progression of disease. Although the hypothesis would undergo significant modification during 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 during the past several decades has facilitated major advances in our understanding of nodal pathways operative within vessel-intrinsic and vessel-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 nonadhesive, nonthrombotic barrier between the flowing blood and underlying tissue. Interendothelial junctions tightly regulate permeability of fluid and macromolecules across the endothelial surface. Control of vasomotor tone occurs via dynamic signaling among bloodborne humoral factors and metabolic products of underlying tissues, and chemical communication between the endothelium and the vascular smooth muscle cells (VSMCs) of the medial layer of the vessel. Central to maintenance of this homeostasis is optimal endothelial function, and laminar shear stress 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.25–29 Anatomically, this correlates to regions long recognized as most prone to the development of atherosclerotic lesions. In the past 5 years, in vivo animal experiments have demonstrated that EC deficiency of KLF2 and KLF4 also predispose to atherosclerosis. In mice deficient in apolipoprotein E (ApoE) 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.31 In vitro and in vivo data demonstrate that both KLF2 and KLF4 are potent inducers of endothelial NO synthase and thrombomodulin expression and also inhibit cytokine-induced expression of adhesion factors, including vascular cell adhesion molecule-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 (eg, ZO-1 and occludin).32,33 In vivo permeability studies on KLF4 are limited thus far; however, KLF4 is likely to influence permeability via transcriptional regulation of vascular endothelial-cadherin.34

Determination of the precise mechanisms by which laminar shear stress 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 laminar shear stress. These nonciliated cells do
not express endothelial NO synthase in response to shear; instead, they lose their cobblestone appearance and acquire a mesenchymal-transitional phenotype. Although 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.

A molecular link between flow and its downstream effects is signaled, in part, by mitogen-activated protein kinase pathways that activate extracellular-signal-regulated kinase (ERK). KLF2 and KLF4 are induced by ERK activation. The KLF2 promoter is upregulated by myocyte enhancer factor-2 binding downstream to both the MEK5/ERK5/myocyte enhancer factor-2 and the AMPK/ERK5/myocyte enhancer factor-2 flow pathways. ERK5-dependent KLF4 induction confers the vasoprotective phenotype described by enhanced expression of antithrombotic, hermotic, and vasodilatory genes.

Post-transcriptional regulation also seems to play a role both in endogenous regulation of KLF2 and KLF4; 2 laboratories independently found that inhibition of miR-92a by atheroprotective flow allows for increased levels of EC KLF2 and KLF4.

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, transforming growth factor-β1, and activin receptor-like kinase-1. In in vitro scratch assays, overexpression of KLF6 leads to more rapid wound healing and enhanced EC migration. KLF11 suppresses NF-κB–mediated EC activation, a role that may be particularly important in diabetes mellitus. 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. In regard to KLF2 and KLF4, however, the data are 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. Therein monocytes differentiate to macrophages, which then take up oxidized low-density lipoprotein and become foam cells; an essential characteristic of the atherosclerotic lesion, manifesting early in disease as a fatty streak. 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.

Myeloid Cells
As factories of both cytokine and protein mediators of inflammation macrophages are central to the development atherosclerosis. There are 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. Although there are important data elucidating the role of KLF2 in acute inflammation (sepsis), because of 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. Animals with myeloid-specific deletion of KLF2 have elevated baseline plasma levels of proinflammatory molecules, including interleukin (IL)-1β and tumor necrosis factor-α.
supporting the idea that KLF2 is a tonic repressor of myeloid activation.\textsuperscript{21} Low density lipoprotein receptor null mice with myeloid-specific KLF2 deletion develop a markedly greater atherosclerotic burden than controls.\textsuperscript{57} Mechanistically, there are data that support a role for myeloid KLF2 deficiency in enhancing macrophage adherence to ECs\textsuperscript{57} and induce oxidized low-density lipoprotein uptake,\textsuperscript{50} thus augmenting macrophage-derived foam cell formation.

\textbf{KLF4} \\
Gain- and loss-of-function studies show that KLF4, as a downstream target of PU.1, promotes monocyte differentiation.\textsuperscript{58} In vivo experiments in KLF4\textsuperscript{−/−} chimeric mice demonstrate a role of KLF4 in inflammatory monocyte differentiation, with KLF4 expression necessary for maturation of both Ly6C\textsuperscript{hi} and Ly6C\textsuperscript{lo} monocyte populations.\textsuperscript{59}

Beyond developmental biology, KLF4 is also a mediator of macrophage subset specification by regulating macrophage M1/M2 polarization.\textsuperscript{65} In response to M2 stimuli (IL-4 and 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 (lipopolysaccharide, interferon-γ), 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 KLF4. Mechanistically, KLF4 promotes the M2 phenotype by cooperation with STAT6 to promote M2 targets. KLF4 inhibits KLF4 expression necessary for maturation of both Ly6C\textsuperscript{hi} and Ly6C\textsuperscript{lo} monocyte populations.\textsuperscript{59}

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Other \textbf{KLFs} \\
Other KLFs, including KLF1, KLF3, and KLF10, are expressed in myeloid cells and may participate in vascular inflammation and atherosclerosis.\textsuperscript{2,26,63}

\textbf{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.\textsuperscript{64} Our group and others have demonstrated a role for KLF2, 4, 10, and 13 in regulation of adaptive immunity, with implications for atherosclerosis.

\textbf{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\textsuperscript{CIP1/WAF1}.\textsuperscript{65,66} In addition, KLF2 serves a major role as a regulator of T- and B-cell survival and migration.\textsuperscript{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, CC chemokine receptor-7, and integrin β7. T cells lacking KLF2 express proinflammatory chemokine receptors, such as CXC\textsubscript{3}, CC chemokine receptor-3, CC chemokine receptor-5, and CD69, on memory cells and activated thymocytes.\textsuperscript{68} These changes may lead to altered homing patterns of naïve T cells to nonlymphoid tissues and attenuation of T-cell proliferation. Although 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.\textsuperscript{69}

\textbf{KLF4} \\
Differentialization of helper T cells type 17 (Th17) and the expression of IL-17 by these cells are regulated by KLF4.\textsuperscript{70,71} Although 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.\textsuperscript{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.\textsuperscript{71} Taken together, these studies suggest a role of KLF4 in regulating T-cell activation and proliferation, which may play into an effect on atherogenesis.

\textbf{KLF10} \\
Several studies have defined an atheroprotective role of regulatory T cells. KLF10 drives CD4+CD25+ T-cell activation and regulatory T-cell differentiation and suppressor function.\textsuperscript{73} Overexpression of KLF10 in regulatory T cells induces expression of transforming growth factor-β1, an atheroprotective cytokine. Importantly, in an ApoE null, scid background, adoptive transfer of KLF10-deficient CD4+CD25+ T cells accelerates atherosclerosis.

\textbf{KLF13} \\
KLF13 was identified initially as a transcription factor expressed in activated T lymphocytes.\textsuperscript{74} KLF13 enhances expression of the proinflammatory chemokine, regulated on activation, normal T cell expressed and secreted, to promote T-cell activation and attenuates promoter activity of a potent antiapoptotic factor, B-cell lymphoma-extra large, to promote T cell and survival.\textsuperscript{75}

\textbf{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.\textsuperscript{14} 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 lipopolysaccharide via attenuation of the toll-like receptor signaling pathway.\textsuperscript{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<sup>WAF1</sup> expression and downregulation of c-Myc and cyclin D2.<sup>78</sup> Immune Cell Infiltration→VSMC Proliferation

In a healthy artery, VSMCs 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 low and they express markers of a contractile cellular phenotype, including smooth muscle α-actin, smooth muscle myosin heavy chain, h-caldon, and smoothelin (reviewed in Klaewsongkram et al). 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), VSMCs 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 VSMCs 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, although details of the changed expression profile have been documented rigorously in vitro. The relocated VSMC may proliferate (neointimal formation), take on characteristics of foam cells, elaborate inflammatory signals, and synthesize extracellular matrix proteins that lead to the development of fibrous plaque cap. 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. The Owens laboratory has demonstrated that oxidized low-density lipoprotein induces both phenotypic switching and enhanced VSMC migration in a KLF4-dependent fashion. Control of VSMC proliferation by KLF4 is mediated via induction of p53 and p21<sup>WAF1</sup>/MIP1, reminiscent of mechanism in other cell types. KLF4 inhibits expression of VSMC differentiation genes by interfering with expression and function of the potent SMC coactivator myo- cardin. Interestingly, KLF4 inhibits myocardin by binding to the promoter in cooperation with NF-κB. Thus, in contrast to ECs, KLF4 is proinflammatory by cooperating with NF-κB in activated. Direct evidence, via VSMC-specific genetic gain- and loss-of-function experiments, that altered VSMC KLF4 alters atherosogenesis is lacking as yet; however, recent studies using miRNA approaches are supportive. MicroRNA-145 is highly expressed in arteries but is attenuated after vascular injury and in atherosclerosis. 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 (among other targets). Of note, VSMC-specific lentiviral overexpression of miR-145 reduces KLF4 levels, limits atherosclerosis, and enhances plaque stability in the ApoE null mouse model.

KLF5

KLF5 expression in VSMCs is induced after vascular injury and in atherosclerotic lesions. Genetic deficiency of KLF5 leads to baseline thinning of the medial and adventitial walls of arteries and inhibition of neointimal proliferation after vascular injury. A potential proatherogenic role for KLF5 is also suggested by the gene profile it activates. Targets include platelet-derived growth factor-A, transforming growth factor-β1, cyclin B, Egr-1, and plasminogen activator inhibitor-1; genes that enhance proliferation, migration and vascular inflammation. 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. 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. 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. 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. Ex vivo (fibrin clot) and in vivo (carotid injury) experiments demonstrate that KLF4 protects against thrombosis, even in the presence of inflammation. EC KLF2 and KLF4 increase thrombomodulin and tissue-type plasminogen activator expression and decrease plasminogen activator inhibitor and cytokine-stimulated tissue factor expression, consistent with an antithrombotic 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. Finally, as mentioned above, miR-145–mediated downregulation of VSMC KLF4 may enhance stability of atherosclerotic plaque.
Table. Summary of Expression and Function of KLFs in Regard to Atherosclerotic Vascular Disease

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>KLF</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>Endothelial cell</td>
<td>KLF2</td>
<td>Promotes anti-inflammatory and antithrombotic phenotypes by inducing eNOS, TM, and tPA and by inhibiting cytokine-induced expression of adhesion factors (eg, VCAM-1 and E-selectin) and PAI-1</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Promotes anti-inflammatory and antithrombotic phenotypes by inducing eNOS, TM, and tPA, and by reducing expression of VCAM-1, E-selectin, and PAI-1</td>
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<td></td>
<td>KLF6</td>
<td>Promotes vascular remodeling (in cooperation with SP1) by inducing endoglin, collagen α1, TGFβ1, and activin receptor-like kinase-1</td>
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<tr>
<td></td>
<td>KLF11</td>
<td>Attenuates NF-κB-mediated EC activation</td>
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<tr>
<td>Immune cell: myeloid cell</td>
<td>KLF2</td>
<td>Tonic repressor of myeloid activation by inhibiting the NF-κB pathway</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Promotes an atheroprotective phenotype</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Promotes an atheroprotective phenotype (as a downstream target of PU1)</td>
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<td></td>
<td>KLF4</td>
<td>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</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Inhibits leukocyte expression of MMPs, implying protection against plaque rupture-mediated thrombosis</td>
</tr>
<tr>
<td>Immune cell: T lymphocyte</td>
<td>KLF2</td>
<td>Maintains T-lymphocyte quiescence by inhibiting c-Myc and by inducing p21CIP1 expression</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Required for the expression of surface receptors and trafficking molecules, such as L-selectin, CCR7, integrin β7, and the sphingosine-1-phosphate receptor S1P1R</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Promotes an anti-inflammatory phenotype in a mouse model of T-cell–dependent myocarditis</td>
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<td></td>
<td>KLF4</td>
<td>Regulates differentiation of Th17 cells and IL-17 production by binding to IL-17 promoter</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Promotes an atheroprotective effect by inducing expression of TGFβ1, by regulating CD4+CD25− T-cell activation, T regulatory cell differentiation and T regulatory cell suppressor function</td>
</tr>
<tr>
<td></td>
<td>KLF4</td>
<td>Promotes T-cell activation by inducing expression of the proinflammatory chemokine, RANTES, and T-cell survival by attenuating BCL-XL promoter activity</td>
</tr>
<tr>
<td>Immune cell: B lymphocyte</td>
<td>KLF3</td>
<td>Regulates B-cell differentiation and quiescence and B-cell proliferative response to LPS via downregulation of toll-like receptor signaling pathway</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Regulates activation-induced B-cell proliferation by inducing p21CIP1 and downregulating c-Myc and cyclin D2</td>
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<tr>
<td>Vascular smooth muscle cell</td>
<td>KLF4</td>
<td>Level is rapidly induced in response to vascular injury</td>
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<tr>
<td></td>
<td>KLF4</td>
<td>Critical for VSMC phenotypic switching between contractile and proliferative states</td>
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<td></td>
<td>KLF4</td>
<td>Enhances VSMCs migration in response to OxlDL</td>
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<td></td>
<td>KLF5</td>
<td>Cooperates with NF-κB to inhibit expression of VSMC differentiation genes</td>
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<tr>
<td></td>
<td>KLF5</td>
<td>Enhances VSMC proliferation, migration, and vascular inflammation by inducing expression of proatherogenic genes (eg, PDGF-A, TGFβ1, cyclin B, Egr-1, and PAI-1)</td>
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<td></td>
<td>KLF5</td>
<td>Level is reduced after vascular injury in mouse models and in human atherosclerotic disease and aortic aneurysms</td>
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<td></td>
<td>KLF15</td>
<td>Loss results in proatherosclerotic and proinflammatory phenotypes in mouse models</td>
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<td></td>
<td>KLF15</td>
<td>Reduces activity of NF-κB on inflammatory gene promoters via direct interaction with p300</td>
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BCL-XL indicates B-cell lymphoma-extra large; CCR, CC chemokine receptor; EC, endothelial cell; eNOS, endothelial NO synthase; FABP4, fatty acid binding protein 4; IL-17, interleukin-17; KLF, Krüppel-like factor; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; NF-κB, nuclear factor-κB; OxlDL, oxidized low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; PCAF, P300/CBP-associated factor; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; S1P1R, sphingosine-1-phosphate receptor; SP1, specificity protein; TGF, transforming growth factor; Th17, helper T cells type 17; TM, thrombomodulin; tPA, tissue-type plasminogen activator; VCAM, vascular cell adhesion molecule; and VSMC, vascular smooth muscle cell.

See text for details and references.

Conclusions

Atherosclerosis is a complex, chronic, highly morbid disease; it is the leading cause of death in the United States as well as increasing parts of the rest of the world. Cardiovascular physicians have sowed of powerful medical therapies that have attenuated the mortality of the disease, yet ≈715,000 Americans experience myocardial infarction each year. KLFs have potent effects on a broad range of vascular processes that contribute to atherogenesis (summarized in the Table), and the cumulative data are sufficient to warrant their consideration as therapeutic targets.

Although 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 components of the Mediterranean diet including broccoli, grapes, red wine (resveratrol), and olive oil enhance KLF4 and KLF2 expression or reduce KLF6 and cigarette smoke increases VSMC KLF4 expression—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 KLF4.110,110,110 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 cocultured VSMCs and reduce atherosclerotic lesion formation in ApoE null mice.113 Hergenreider et al113 exposed ECs to shear stress or lentivirus-mediated overexpression of KLF2. Extracellular vesicles secreted from these cells were enriched in the mir143/145 and were atheroprotective. This is exciting news for fans of the KLFs and of great interest for all those fascinated by communication between ECs and VSMCs. We are confident that Dr Ross would continue to find this topic utterly enthralling.

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**Disclosures**


ATVB Named Lecture Review—Insight into Author

ATVB Named Lecture Reviews—Russell Ross Memorial Lectureship in Vascular Biology

Insight into the Author: Mukesh K. Jain, MD, Case Western Reserve University

How did you choose the profession of being a physician-scientist?

I have been interested in medicine since grade school. My initial exposure to research began during high school and, since that time, I have spent nearly every summer through the first 2 years of medical school in various laboratories. I considered entering an MD, PhD program but, truth be told, felt unsure of myself compared with the brilliant students that entered these programs. However, the desire to engage in science continued to smolder. As a medical resident, I worked with Kathleen Morgan at the Beth Israel Hospital, Boston—work that culminated in a first author paper. This positive experience along with encouragement from my wife (who had become increasingly fatigued listening to me vacillate over engaging in research) led me to join Edgar Haber’s laboratory in 1994. This is also where I met Mu-En (Arthur) Lee—at the time, a young faculty member in the cardiovascular program led by Haber. The combined influence of these 2 exemplary physician-scientists solidified my confidence and affirmed my passion for scientific investigation.

Who have been your role model(s) in your scientific and professional life?

Mu-En (Arthur) Lee. When I joined the Haber laboratory in 1994, I met Arthur. Although I was formally a Haber fellow, I bonded with Arthur and became a member of both laboratory groups. Arthur was intense, incredibly hard working, and passionate about discovery—it was infectious! That said, what I admired most about Arthur was the coupling of these qualities with the highest level of integrity and generosity. He was a wonderful role model and I very much wished to be like him. Regrettably, we lost Arthur to illness much too early—in April of 2000 at the age of 46 years. This saddens me deeply as I never had the opportunity to thank him appropriately for his enormous impact on my professional life.

How have mentors contributed to your professional development?

I firmly believe one needs mentors at every career stage. I have had the good fortune to have many individuals favorably enrich my professional career and regret that I cannot name them all. Drs Haber and Lee were the dominant scientific influence during my training. In regard to my clinical education, I wish to acknowledge Patrick T. O’Gara, Director of Clinical Cardiology at the Brigham and Women’s Hospital. I was Dr O’Gara’s fellow in clinic. It was in this setting that I began to truly appreciate that the practice of medicine cannot be learned from a textbook, it is truly an art, and Pat is a master of the craft. I still feel privileged to have observed and learned from him first-hand.

During my transition to faculty, critical mentors were Peter Libby, Thomas Michel, and Daniel I. Simon. After relocating my clinical training in cardiovascular medicine, I rejoined Arthur Lee’s laboratory for additional research training. However, Arthur’s death four months later was devastating and the future seemed uncertain. I met with Dr Libby and, for reasons that still escape me, he offered support so I could initiate an independent laboratory. I was astounded as I was certain that several more years of mentored training were needed. However, I readily accepted his offer and did not share my insecurities with Dr Libby until many years later! This was a pivotal moment in my career and I remain deeply grateful for being provided such a wonderful opportunity.

Not surprisingly, the initial years establishing a laboratory were challenging. Fortunately, my research space in the Thorn Building was in proximity to Drs Simon and Michel who were exceptionally supportive. Thomas taught me the basics of running a laboratory and invited me to participate in joint laboratory meetings with him and 2 other laboratories (Drs Koren and Mendez). These weekly meetings provided a robust forum for scientific exchange. Dana’s laboratory was directly across the hall from me and we developed rich and long-standing research collaborations. Perhaps, even more important was his willingness to serve as a big brother and offer advice on essentially any topic—whether it be related to pursuing a scientific question, developing successful collaborations, balancing work and family, dealing with publication and funding rejections [and there were many!], and much more. We developed a close friendship, one that has been enormously influential in my career and a major reason that I moved with him to Case Western Reserve University (CWRU) University Hospitals Case Medical Center (UHCMC) in 2006. At CWRU/UHCMC, I met 2 additional individuals who, along with Dr Libby, have served as wonderful mentors. Jonathan Stamey and Richard A. Walsh. Jonathan is an exceptional scientist and intellect whose input has inspired me to push scientific boundaries and encouraged me to more vigorously pursue translational efforts. Dr Walsh, our Chairman of Medicine, has provided unwavering support for the program, critical insights on leadership, and sage advice on balancing administrative responsibilities with one’s own career aspirations.

What have been important influences on your professional life?

In addition to mentors, I would say family, colleagues, and trainees. As the son of immigrants, core values such as commitment, hard work, and perseverance were instilled early and have served me well. In addition, the unconditional support and love of my family (especially my mother) has been essential in allowing me to weather the ups and downs of academic life. I have also had the good fortune to work with and learn from wonderful colleagues at every career stage, whether it be during my training (Amit N. Anand, David Zhao), research training (Nicholas E.B. Silbina, Hong Wang, Cam Patterson, Chung-Ming Hosh, Koji Mameura, Mark W. Frennenberg, and Michael T. Chin), and as a faculty member (James C. Fang, Marco A. Costo). Finally, I have been fortunate to attract numerous and talented trainees whose abilities often exceed my own and from whom I have learned much during the years.

What wisdom do you impart on new investigators?

1. Read often, read broadly, and attend conferences or seminars outside of your research area. These exposures will stimulate new ideas and approaches that can be applied to key unanswered questions in your area of interest.
2. Once you have identified an important problem, focus on this intensively. Although everyone loves to hit a homerun with every publication, remember that hitting singles is OK as long as you make important contributions. OK is better than nothing.
3. Life as a young investigator is hard, so build a good support network of junior and senior colleagues whom you trust. Also, although science has traditionally been an individual sport, collaborative interactions are important (increasingly so as we enter the era of team science). In these interactions, be generous and flexible as you are likely to gain more by working collaboratively than alone. And perhaps most critically, remember that the scientific community is small, so be good to your colleagues and mentors—do not burn bridges! Treat colleagues with integrity and respect—failure to do so is karmically unacceptable and ultimately to your detriment. Cherish these relationships as they are important and will serve you well during the vicissitudes that typify academic life.
4. Encourage, support, and be generous with your trainees. Lead by example. Their success is key as they will ultimately be your most important contribution to the profession.
5. Be resilient. Funding lines are low and publishing can feel like a chore so it is easy to become discouraged. Just remember that being a scientist is one of the best jobs in the world. You can ask and answer questions about the nature of life that are important to you—and get paid to do it!

Which direction do you envisage your science and professional career taking?

There is a thrill in the discovery of fundamental biological processes that is unparalleled and I hope to continue to contribute to this endeavor in a meaningful way. I would also like to become much more engaged in translational efforts to determine whether some of the insights gleaned from our basic work can be exploited to create therapies that positively affect human health. Beyond my own laboratory, I am involved in several efforts that are particularly gratifying. I serve as Scientific Director of the Harrington Discovery Institute, a national initiative founded by Dr. Stamey that is dedicated to helping the nation’s most promising physician-scientists advance breakthrough discoveries into medicines for the benefit of patients and society. I also have a leadership role in the American Society for Clinical Investigation (ASCI), one of the oldest honorific societies for physician-scientists. I will serve as President of the ASCI 2014–2015 and hope to use this platform to enhance support for biomedical research, encourage, and facilitate trainee involvement in research and help sustain the academic careers of investigators. Long-term, I hope to remain actively engaged in academic leadership to promote and support research, education, and training efforts at the institutional and national levels.

What sports do you follow?

I am an avid fan of professional football and tennis. Sadly, my hometown Buffalo Bills continue to go through growing pains.

What are your favorite foods and are they heart healthy?

When I first moved to the United States at the age of 6 years, my favorite food was french fries. This has not changed in the ensuing 42 years. Much to my chagrin, french fries are not heart healthy and they make me grow in the wrong direction!
Regulation of an Inflammatory Disease: Krüppel-Like Factors and Atherosclerosis
Mukesh K. Jain, Panjamaporn Sangwung and Anne Hamik

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