Vascular Implications of the Krüppel-Like Family of Transcription Factors

Toru Suzuki, Kenichi Aizawa, Takayoshi Matsumura, Ryozo Nagai

Abstract—The Krüppel-like factor (KLF) family is a recently highlighted group of zinc finger transcription factors given their important biological roles which include the vasculature. KLF2, KLF4, KLF5, and KLF6 are notable factors that have been implicated in developmental as well as pathological vascular processes. In this brief review, we provide an up-to-date summary of the physiological functions and cellular effects as well as transcriptional regulatory mechanisms of the vascular KLFs. Through such, we aim to provide a working view for understanding the pathological actions of KLFs in the vasculature. (Arterioscler Thromb Vasc Biol. 2005;25:1135-1141.)

Key Words: transcription ■ gene expression ■ zinc finger ■ Krüppel-like factor

The mammalian Krüppel-like factor (KLF) family of zinc finger transcription factors has recently received increased attention. There are >15 known family members at present, and despite initial expectations that they would have redundant functions, they, in fact, have individually important biological functions, as shown by gene knockout studies (eg, KLF2, KLF4, and KLF5). A number of these factors show developmental and pathological implications in the vasculature. This brief review focuses on the subset of these factors expressed in the vasculature and discusses their functional roles as well as regulation to provide a working view to understand their unique functions and actions. We apologize in advance that we limit our discussion to the vascular KLFs and that not all factors are discussed in detail in the present brief review because of length restraints but refer the interested reader to a number of excellent reviews in recent years that have covered different aspects of the KLFs.1–8 We also provide a Table, which summarizes their known functions.

Eukaryotic Zinc Finger Proteins
We begin with a brief introduction to zinc finger transcription factors. The zinc finger motif, which characterizes the zinc-finger-type transcription factors, is one of the most common motifs in the eukaryotic cell being found in proteins ranging from enzymes to transcription factors. The paired cysteine and histidine-type (C2H2) zinc finger motif, identified ~2 decades ago,9 is the focus of attention of the present review, but other zinc finger motifs, such as those that contain 4 cysteines (C4), are found in nuclear receptors (eg, estrogen receptor and retinoic acid receptor [RAR]) as well as GATA-type transcription factors. The cysteine and histidine residues are important to spatially coordinate and anchor the zinc atom.

Comparison of the human genome with that of the yeast genome shows that transcription factors with the zinc finger motif have evolved tremendously in parallel with the increased genomic complexity.10 The selective increase of C2H2-type zinc finger transcription factors in higher eukaryotes likely reflects the need to diversify to accommodate for acquired biological functions (eg, development and differentiation). Among the C2H2-type zinc finger transcription factors are well-known cellular transcription factors such as early growth response factor-1 (Egr-1)11 and specificity protein-1 (Sp1),12 in addition to the KLFs. Therefore, the KLFs likely evolved as a subgroup of zinc finger transcription factors in response to acquired biological diversity.

Krüppel-Like Factors
The KLFs have in common 3 contiguous C2H2-type zinc fingers at the carboxyl terminus that comprise the DNA-binding domain.1–8 “Krüppel,” which means “cripple” in German, is the name of the founding Drosophila factor.13 The “Krüppel-like family” designation was first used to distinguish between similar C2H2-type zinc finger factors into 2 groups, namely those similar to Krüppel and those to GLI (for glioblastoma). The KLFs contain a signature consensus amino acid finger sequence ([Y/F] XCX2CXX3FX5LX2HXRXHTGEKP) that is still used today.14 KLFs are also very similar to Sp1 and its family members in their zinc fingers. Sp1 was one of the first eukaryotic transcription factors identified as a protein that stimulates transcription of the SV40 early promoter15 and is still used today as a benchmark for understanding
<table>
<thead>
<tr>
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<td>Erythropoiesis Differentially regulates interleukin-12 p40 expression depending on the cellular status Important implications for the control of inflammation Essential for the expression of the adult β-globin gene and erythrocyte differentiation</td>
<td>Activator</td>
<td>p300/CBP P300 PCAF SWI/SNF mSin3A</td>
<td>19q13</td>
<td>Modified EKLF binds to the defective delta-globin promoter and enhances delta-globin gene expression to increase HbA2 levels and inhibit HbS polymerization Potential treatment of sickle cell patients A silent β-thalassemia mutation in the distal CACCC box affects the binding and responsiveness to EKLF</td>
</tr>
<tr>
<td>KLF2</td>
<td>LKLF</td>
<td>Lung, blood vessels, lymphocytes Endothelial cells</td>
<td>Blood vessel, lung, development, T-cell survival Essential for late stages of normal lung development Targeted disruption causes embryonic lethality due to hemorrhage and abnormal vessel morphology Reduced expression at aortic branch points Expression induced by laminar flow and inhibited by inflammatory cytokines Overexpression in human umbilical vein endothelial cells induces endothelial NO synthase, inhibits cytokine-induced vascular cell adhesion molecule-1 expression, and prevents flow-mediated leukocyte adhesion</td>
<td>Activator</td>
<td>WWP1 CBP/p300</td>
<td>19q13</td>
<td>Regulated by shear stress Speculated to be instrumental for the communication between endothelial cells and smooth muscle cells</td>
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<td>KLF3</td>
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<td>Erythroid tissue and brain-enriched</td>
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<td>KLF4</td>
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<td>Gut-enriched Vascular smooth muscle cells Endothelial cells</td>
<td>Antiproliferation, survival Expressed in vascular endothelial cells, induced by laminar flow Represses TGF-β dependent induction of SM22α and α-smooth muscle actin Vascular phenotype not reported in targeted mice</td>
<td>Activator</td>
<td>Repressor p300/CBP</td>
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<td>Reduced in adenomas of patients with hereditary adenomatous polyposis in the gut</td>
</tr>
<tr>
<td>KLF5</td>
<td>BTEB2</td>
<td>Epithelial tissue Vascular smooth muscle cells Gut-enriched</td>
<td>Cell growth Induced in activated smooth muscle cells (eg, vascular injury) KLF5 homologous die at an early embryonic stage Induces cell proliferation when forcibly expressed and induces focus formation consistent with its suggested role as a protooncogene KLF5 heterozygotes show reduced arterial wall thickening, cardiac hypertrophy and angiogenesis in response to external stress</td>
<td>Activator</td>
<td>p300/CBP SET NF-κB p50 RARα</td>
<td>13q21</td>
<td>KLF5 heterozygotes show reduced responses to vascular injury and tumor implantation, reduced responses to vascular injury and tumor implantation, reduced angiotensin II–induced cardiac hypertrophy and fibrosis, gastrointestinal abnormalities, and diminished type IV collagen expression in the subendothelial An association with breast cancersuggested</td>
</tr>
<tr>
<td>KLF6</td>
<td>GBF</td>
<td>Ubiquitous Endothelial cells Liver cells</td>
<td>Putative tumor suppressor Induced after vascular injury and activates latent TGFβ1 Cooperates with Sp1 to activate endoglin, collagen α1(I), urokinase-type 1 promoters Activates inducible NO synthase under stress conditions Upregulated during early hepatic fibrosis Inhibit cell growth by upregulating p21</td>
<td>Activator</td>
<td>Unknown</td>
<td>10p15</td>
<td>Thought to be a factor induced in endothelial cells by vascular injury that activates TGF-β, TGF-β signaling receptors and TGF-β–stimulated genes and urokinase plasminogen activator implicated to be a tumor suppressor protein mutated in a variety of tumor states, including prostate cancer</td>
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<tr>
<td>KLF7</td>
<td>UKLF</td>
<td>Ubiquitous</td>
<td>Cell–cycle arrest</td>
<td>Activator</td>
<td>Unknown</td>
<td>2q32</td>
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<tr>
<td>KLF8</td>
<td>BKLF3</td>
<td>Ubiquitous</td>
<td>Knockout: required for murine growth; negative regulator of proliferation of myeloid cells</td>
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<td>CBP2</td>
<td>Xp11</td>
<td>Unknown</td>
</tr>
<tr>
<td>KLF9</td>
<td>BTEB1</td>
<td>Ubiquitous</td>
<td>Neurite outgrowth and carcinogen metabolism Functionally relevant to progesterone receptor -interacting protein</td>
<td>Activator</td>
<td>mSin3A</td>
<td>9q13</td>
<td>Unknown</td>
</tr>
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general mechanisms of transcriptional regulation. At present, the KLFs, along with the Sp1-family of factors, are often collectively called the Sp and KLF family of zinc finger transcription factors. Factors of the Sp subset have 6 to 8 members, whereas the KLF subset has ≈16 members. The KLFs, when identified, were often given individual names such as EKLF (erythroid KLF), LKLF (lung KLF), etc; but at the completion of the human genome project, a letter was sent to researchers who had isolated KLFs asking for an international collaboration to unify the nomenclature by a numbering system based on chronological order of identification. Thus after, the KLFs were given numbers that are often used side by side with the original name or the numbering system alone. Hereafter, the numbered names will be used primarily in this review. Among the notable KLFs are erythroid differentiation factor KLF1 (EKLF),15 which was the first mammalian factor identified, and the tumor suppressor gene KLF6 (GBF/Zf9/COPEB), which we and others identified as a cellular factor possibly involved in HIV-1 transcriptional regulation.16,17 Importantly, as will be described in detail hereafter, KLF2 (LKLF), KLF4 (GKL/EZF), KLF5 (BTEB2/IKLF),18,19 and KLF6 (GBF/Zf9/COPEB) are of particular importance in the vasculature. KLF5 is of prominent importance as a key regulator of cardiovascular remodeling in response to stress, as shown by our murine knockout studies.20

**Vascular Roles of KLFs**

Studies to date have established that KLF2, KLF4, KLF5, and KLF6 harbor important roles in the vasculature, and that they likely have functional implications in developmental as well as in pathological conditions. Before continuing further on the role of mammalian KLFs, we first note a study done in zebrafish, which is a model organism often used to understand the ancestral and systematic role of factors in vertebrates. This study showed that the KLFs are in fact a family of factors important in blood vessel development in addition to hematopoiesis and epidermal development.21 Although the homologues of KLF2 and KLF4 in particular showed developmental implications, this study demonstrated the functions and ancestral origins of the KLF family and importantly suggested that the KLFs have evolved with preference in functional implications for blood and vessel development in vertebrates.

Returning to mammals, the functional role of KLF2, as shown by genetic knockout studies in mice, showed that it is important for vascular development.22 KLF2 is expressed in vascular endothelial cells (ECs) in the embryo, and null mutants show normal angiogenesis and vasculogenesis. However, null embryos die in utero because of hemorrhaging as a result of defective blood vessel morphology. That is, they show thin tunica media and aneurysmal dilatation in the veins and arteries, and aortic vascular smooth muscle cells (SMCs) are malformed (cuboidal morphology) and fail to organize into compact tunica media. EC necrosis, reduction in the number of vessel wall pericytes and differentiating SMCs, and decreased deposition of extracellular matrix are also seen. These findings suggest that ECs may regulate the assembly of the vascular tunica media and concomitant vessel wall stabilization during mammalian embryogenesis. Pathologically, KLF2 is uniquely induced by steady laminar flow in the endothelium.23 KLF2 expression has also been shown to be inhibited by the inflammatory cytokine interleukin-1β in cultured ECs. Overexpression of KLF2 induces endothelial NO synthase expression and total enzymatic activity in addition to inhibiting the induction of vascular cell adhesion molecule-1 and endothelial adhesion molecule E-selectin in response to various proinflammatory cytokines. These data collectively implicate KLF2 as an antiatherosclerotic and anti-inflammatory regulator of endothelial activation in response to proinflammatory stimuli.24

KLF4 has also been knocked out, but a vascular phenotype has not been reported. KLF4 is highly expressed in the
differentiating layers of epidermis, and null mice die shortly after birth because of loss of skin barrier function, suggesting that KLF4 is important for barrier acquisition. KLF4 was also isolated as a KLF expressed in the vasculature and is induced by shear stress. KLF4 has been shown to repress transforming growth factor-β (TGF-β)–dependent increase of SMG differentiation marker genes, including α-smooth muscle actin and SM22α. A recent study showed that KLF4 represses myocardin-induced activation of SMG genes and expression of myocardin itself. Although KLF4 is not normally expressed in differentiated SMCs, it is upregulated by platelet-derived growth factor (PDGF)-BB–treated cultured SMCs and in response to vascular injury in vivo. Therefore, KLF4 may be a key effector of induced phenotypic switching of SMCs. Collectively, it is most likely that KLF4 is a pathologically induced factor in ECs as well as in vascular SMCs to regulate vascular cell function.

The role of KLF5 in the vasculature first received attention when it was isolated as a transcription factor that binds the promoter of the embryonic smooth muscle myosin heavy chain SMem gene. KLF5 activates many genes inducible during cardiovascular remodeling, such as PDGF-A/B, Egr-1, plasminogen activator inhibitor-1, inducible NO synthase, and vascular endothelial growth factor receptors. KLF5 is abundantly expressed in embryonic SMCs and is downregulated with vascular development, but importantly, it is induced in proliferating neointimal SMCs in response to vascular injury. In KLF5 gene–targeted mice, homozygotes die at an early embryonic stage, whereas heterozygotes are apparently normal. However, in response to external stress, the arteries of heterozygotes exhibit diminished levels of SMCs and adventitial cell activation. KLF5 activities are regulated by a variety of transcriptional regulators and nuclear receptors, such as RARs. Interestingly, an RAR agonist suppresses KLF5 and (cardio)vascular remodeling, whereas an RAR antagonist activates KLF5 and induces angiogenesis. These results indicate that KLF5 is an important transcription factor in (cardio)vascular remodeling and can be a therapeutic target for (cardio)vascular disease.

KLF6 was independently cloned by a number of groups, including ours. A null mutation has not been published, but its function is generally thought to reflect that of an early growth response factor induced by a variety of stimuli. At present, vascular injury induces this factor in ECs to activate its downstream genes, including TGF-β, TGF-β signaling receptors, and TGF-β–stimulated genes and urokinase plasminogen activator. KLF13 and KLF15 have also been implicated in the vasculature, but these are still early results.

To summarize, several KLFs are expressed in the vasculature and have developmental as well as pathological implications. Taking into consideration the supportive data shown by experiments in zebrafish that KLFs play a developmental role, at least a subset of KLFs most likely play an essential role in vascular development. Importantly, the KLFs play a key role in vascular pathological processes as well. A common property of the KLFs in vascular pathology is that they are induced in response to pathological stimuli (KLF2, KLF4, KLF5, KLF6). Inducibility is a distinguishing feature of vascular KLFs and is vital to understanding their functional roles and differences, as is discussed in the following section on cellular functions. Future studies using conditional vascular knockouts will likely provide a better understanding of their functional roles in the vasculature.

Differential Cellular Effects of KLFs

Given that vascular KLFs are often induced under pathological conditions, their cellular effects, or, that is, their different cellular effects, need to be addressed to understand how their expression affects cellular function. Here, it helps to know that KLFs are often (proto)oncogenes or tumor suppressor genes and thus show effects accordingly on cell growth and proliferation, with the former showing stimulatory and the latter inhibitory effects. Their induced expression thus likely imparts either a stimulatory or inhibitory effect on cell growth. Studies using NIH 3T3 cells showed that KLF4 is not expressed in proliferating cells and is preferentially expressed in growth-arrested states, but when cells are induced to enter the cell cycle, a decrease in expression levels are seen. Forced expression of KLF4 inhibits DNA synthesis in COS-1 cells. In the gut, KLF4 expression is reduced in adenomas of patients with hereditary adenomatous polyposis. Although these observations derived from nonvascular cell lines are not applicable directly to vascular SMCs that show diverse phenotypes (eg, proliferating, growth-arrested, differentiated, and dedifferentiated), KLF4 has been implicated to possess cell growth inhibitory effects. Similarly, KLF6, which also shows characteristics of an early response gene, also inhibits cell growth by upregulating p21 and has been implicated to be a tumor suppressor protein mutated in a variety of tumor states, including prostate cancer. In contrast, KLF5 has been shown to induce cell proliferation when forcibly expressed and to induce focus formation consistent with its suggested role as a proto-oncogene. An association with breast cancer has been suggested. Collectively, although it is still too early to present a unified understanding of the cellular roles of KLFs on the basis of the limited studies addressing this subject because many of them are developmentally regulated factors that are (proto)oncogenes or tumor suppressor factors that show direct effects on cell growth and proliferation when reinduced in pathological states (as confirmed by their association with various oncogenic states), their roles in the vasculature are likely critically dictated by their induction by pathologic stimuli with ensuing cellular effects.

Transcriptional Regulatory Mechanisms

Not only is their regulated expression important for the cellular functions of KLFs, but also the mechanisms of action as a transcription factor also need to be understood. The paired C2H2-type zinc finger is a DNA-binding motif, and the KLFs bind similar GC-rich sites or CACCC-boxes. The sequence specificities of the DNA-binding activity of all of these factors have not been examined. However, well-studied crystal structure analyses of DNA-binding zinc finger transcription factors have allowed the prediction of the cognate DNA-binding sequence from the primary amino acid structure but because these critical amino acids are highly conserved among Sp/KLF zinc finger transcription factors, it
is likely that they share similar DNA-binding properties at least in vitro. It is generally thought that this family of factors binds similar GC-rich sequences in a sequence-specific manner with a selectivity that does not allow individual factors to be clearly discriminated on the basis of DNA-binding characteristics alone. However, it is important to note here that DNA-binding characteristics likely differ in vivo. For example, an experiment using transgenic mice showed that KLF1, but not Sp1, preferentially binds to the \( \beta \)-globin locus site in vivo, despite the fact that KLF1 and Sp1 bind to the locus in biochemical studies in vitro.\(^\text{46}\) We have also shown that the KLF5-binding element in the SMemb gene also binds Sp1 in vitro.

Then how do members of the KLF family exert their individual biological functions in vivo given their similar DNA binding selectivities in vitro? A possible mechanism is through differential regulation by interaction with cofactors and modifications (eg, phosphorylation, acetylation, etc), which results in additional regulation affecting the specificity of actions of KLFs. This is a recent topic of interest that we have been investigating rigorously. First, a common feature of some KLFs is interaction with the coactivator/aceylase p300 and its relative cAMP response element binding protein-binding protein (CBP). CBP and p300 also coactivate the transcriptional activity of KLF5.\(^\text{42,47}\) We have shown that p300 acetylates KLF5 and that acetylation is required for its transactivation by p300 as well as its cell growth stimulatory effects. However, of note, we have also shown that KLF6 is not acetylated by p300,\(^\text{48}\) which is an important example of the different ways of interaction among KLFs that could explain their different biological functions and responses to various stimuli.

Further, these findings led to our recent studies, which showed that an oncogenic regulator, SET, noncatalytically inhibits acetylation of KLF5 by p300 as well as inhibits KLF5-induced cell growth in addition to transactivation.\(^\text{42}\) We have also shown that a deacetylase (histone deacetylase 1 [HDAC1]) negatively regulates the transcriptional activity of KLF5 through direct interaction as well as inhibition of its interaction with p300.\(^\text{49}\) SET and HDAC1 act to negatively regulate transcription of the KLF5 downstream gene PDGF-A chain. Because p300 is induced and SET is repressed by pathological stimulus (eg, phorbol ester) and HDAC1 shows constitutive expression, a transcriptional mechanism involving positive regulation by p300 and negative regulation by SET and HDAC1 with coupled interaction and modification (acetylation) is envisioned, which would affect transcriptional regulation involving KLF5 under pathological states (Figure).

We have also shown that KLF5 associates with the p50 subunit of nuclear factor \( \kappa B \) (NF-\( \kappa B \)) in phorbol ester--induced pathologic conditions in SMCs and that this mechanism is responsible for the delayed yet persistent activation of PDGF-A chain by KLF5 after the initial activation by Egr-1 as mediated by a novel specific interaction with the inducible p50 NF-\( \kappa B \) subunit.\(^\text{31}\) These observations collectively suggest that differential use of cofactors according to different conditions is an important manner in which the functions of KLFs are modulated. This is indeed rational because zinc finger transcription factors often are regulated by protein–protein interaction by associating among themselves as homodimers or heterodimers as well as other cofactors through the zinc finger domain.\(^\text{50}\) Given these findings, it is tempting to assume that protein–protein interaction as well as modification by cofactors play an important role in regulating the KLFs in pathological states. Comprehensive analysis showing differential use of interacting proteins or modifications will add to our understanding of how the actions of KLFs are modulated.

Finally, a point of interest to note is that the KLFs and the relative Sp1 have in common the property to interact with chromatin-associated factors. In fact, the Sp/KLFs, aside from histones, are the only family of DNA-binding factors known to interact with all 3 types of chromatin-remodeling factors, including chemical modification enzymes (eg, acetylases and deacetylases) as well as ATP-independent (eg, histone chaperones) and ATP-dependent (eg, Swi/Snf) nucleosome-remodeling enzymes.\(^\text{8,42,51,52}\) Therefore, it is tempting to envision given that the zinc finger transcription factors are the most widely evolved family of transcription factors in eukaryotes, biological diversification coupled with the emergence of chromatin was a necessary process to further allow for efficient use and access to the tightly packaged DNA genetic information. Elucidating the regulatory pathway involving chromatin-associated factors may be the key to understanding the biological role and regulation of KLFs as well as for other transcription factors.\(^\text{52}\)

**Perspective**

To understand the collective regulation of KLFs will require a more comprehensive analysis of their interactors and modifications coupled with analysis of their cellular functions and implications. Through such, we will be able to better understand the individual as well as combinatorial role of KLFs in regulating cellular processes in the vasculature. We mention combinatorial regulation because often KLFs can be coexpressed and coinduced in the same cell. At times, factors with seemingly opposing actions such as KLF4 and KLF5 are coexpressed. Unfortunately, we do not have information on the further cascade of events associated with these factors in the cell to allow us to determine whether they act on
independent or inter-regulated pathways. Because KLFs often prefer to act in cooperation with interacting proteins (eg, dimers and cofactors), we believe that the actual actions of KLFs are dictated not only by their expression and presence but also by the simultaneous regulation of its regulatory interactor. This would explain the seemingly contradictory coexpression of KLFs with opposing functions. Interaction may be regulated by modification of the protein (eg, phosphorylation and acetylation), which, in turn, may act as a switch to change the interacting protein. How extracellular signaling stimuli and pathways affect these modifications in the cell will likely be an important aspect that will help in understanding the cellular regulation of these factors. A comprehensive analysis of interactors and modifications under pathological conditions will be necessary to better understand the roles of the KLFs in vascular pathologies.

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References


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