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Review Article

Protective Effects of Krüppel-Like Factor 4 against Cardiovascular Disease

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Abstract

Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor implicated in cellular differentiation and proliferation in multiple cell types. Results of recent studies have shown that KLF4 is expressed in the heart and the vasculature, and it plays a key role in the development and progression of cardiovascular diseases. For example, KLF4 inhibits cardiac hypertrophy in multiple murine models. Loss of endothelial *Klf4* in mice results in enhanced atherosclerotic lesion formation. Moreover, deletion of the *Klf4* gene accelerates neointimal formation following vascular injury. This article succinctly reviews the functions of KLF4 in cardiovascular diseases.

ABBREVIATIONS

KLF4: Krüppel-like factor 4; EC: endothelial cell; SMC: smooth muscle cell; Nppa: atrial natriuretic factor; Nppb: b-type natriuretic peptide; Myh7: β-myosin heavy chain; SRF: serum response factor; HDAC: histone deacetylase; CDKN1A: $p21^{WAF1/Cip1}$; CDH5: VE-cadherin.

INTRODUCTION

Krüppel-like factor 4(KLF4) is a zinc-finger transcription factor involved in cellular differentiation and proliferation during development and in various diseases [1-4]. It is also known as gut-enriched KLF, GKLF [5], or epithelial zinc finger, EZF [6]. It is abundantly expressed in the colon, testis, lung, as well as the cardiovascular system [5-10]. Indeed, KLF4 is expressed in the heart from late embryonic development through adulthood [7]. In the vasculature, KLF4 is constitutively expressed in endothelial cells (ECs) [8], while it is induced in phenotypically modulated smooth muscle cells (SMCs) following vascular injury [9,10]. It has also been shown to be expressed in smooth muscle progenitor cells in the arterial adventitia [11]. Results of recent studies by multiple laboratories, including our own, have revealed that KLF4 plays a protective role in various cardiovascular diseases. This review article will succinctly summarize the contribution of KLF4 to cardiovascular diseases by focusing on *in vivo* studies, and provide the update of our knowledge regarding the mechanisms of the action of KLF4.

KLF4 protects against cardiac hypertrophy

Although KLF4 is detectable in the heart [7], precise cell types where KLF4 is expressed have been undetermined yet. At least, it is expressed in cardiomyocytes and ECs in coronary vessels. Analyses of the phenotype of cardiomyocyte-specific *Klf4* knockout mice have revealed the protective role of KLF4 against multiple models of cardiac hypertrophy [12, 13]. Cardiomyocyte-specific *Klf4*-deficient mice are born at the expected Mendelian ratio, and are grown to the adult without any abnormalities in body weight, blood pressure, and the heart rate. However, of interest, they exhibit accelerated cardiac hypertrophy in response to chronic infusion of β-adrenoceptor agonist, isoproterenol, or transverse aortic banding (Figure 1). In response to these hypertrophic stimuli, the enlargement of individual cardiomyocyte is exacerbated and the expression of fetal cardiac genes, such as *atrial natriuretic factor* (*Nppa*), *b-type natriuretic peptide* (*Nppb*), and *β-myosin heavy chain* (*Myh7*), is exaggerated in the hearts of cardiomyocyte-specific *Klf4*-deficient mice, as compared to control mice.

Mechanistic studies have demonstrated that KLF4 suppresses the expression and the activity of myocardin in the heart *in vivo*, as well as in cultured cardiac cells [13]. Myocardin is a very potent transcriptional co-factor for serum response factor (SRF), and it activates the transcription of multiple CArG element-containing fetal cardiac genes, such as *Nppa*, *Nppb*, and *Myh7*, by enhancing the binding of SRF to CArG elements [14]. KLF4 represses the expression of myocardin, and also decreases the binding affinity of SRF to CArG elements, thereby blocking the induction of fetal cardiac genes [13]. The myocardin-SRF complex induces the transcription of multiple CArG element-containing fetal cardiac genes by gathering these elements closely each other, but the KLF4 binding site within the *Nppa* promoter is located between two CArG elements, for example. Binding of KLF4 with the corresponding KLF4 binding site between CArG elements is able to inhibit myocardin-dependent transcription of fetal cardiac genes efficaciously. Recently, multiple histone deacetylase (HDAC) inhibitors, including trichostatin A, valproic

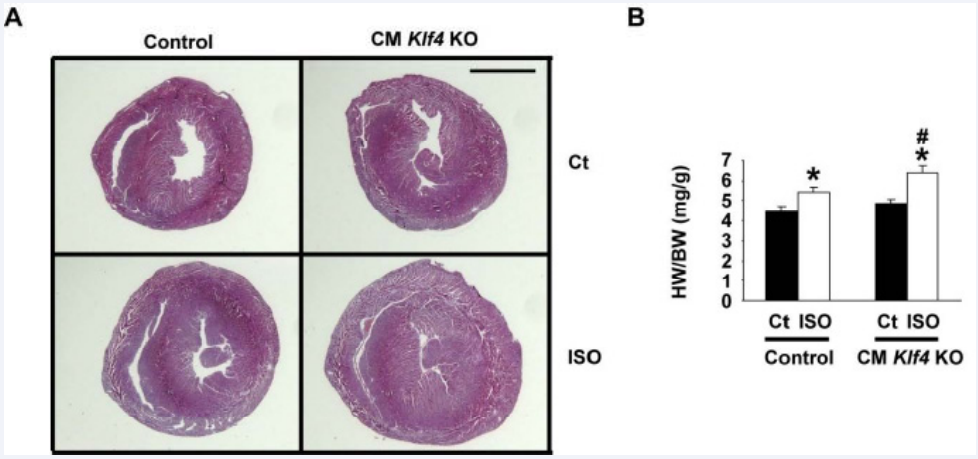

Figure 1 Deletion of *Klf4* accelerates isoproterenol-induced cardiac hypertrophy.
Cardiomyocyte-specific *Klf4* knockout (CM *Klf4* KO) mice and control mice received a continuous infusion of isoproterenol (ISO) or saline (Ct) for 14 days. Cardiac hypertrophy was enhanced in CM *Klf4* KO mice, as compared to control mice.
A: Representative cross-sections of the hearts. Bar 2 mm.
B: The ratio of heart weight to body weight (HW/BW). *P<0.05 compared with ISO-untreated mice. #P<0.05 compared with ISO-treated control mice. Adapted from Yoshida et al. [13], © the American Society for Biochemistry and Molecular Biology.

acid, SK-7041, and Scriptaid, have been shown to prevent cardiac hypertrophy [15, 16]. Of importance, KLF4 mediates anti-hypertrophic effect of HDAC inhibitors by associating with HDAC2 [13, 17]. As such, KLF4 plays a protective role in cardiac hypertrophy by modulating myocardin expression and activity.

In contrast to the phenotype of cardiomyocyte-specific *Klf4* knockout mice which grow up normally under the regular condition, cardiac and smooth muscle-selective *Klf4* knockout mice exhibit the postnatal death and severe growth retardation [7]. These mice are generated by the breeding of transgenic mice expressing Cre recombinase under the control of the *Tagln* promoter and *Klf4* floxed mice. Although the *Tagln* promoter is active in both SMCs and embryonic cardiac cells [18], the phenotype is likely to be caused by the deletion of the *Klf4* gene in cardiac cells, but not in SMCs, based on the following reasons: 1) KLF4 is not normally expressed in SMCs during development; and 2) no differences in the expression levels of SMC differentiation markers and in the morphology of the aorta and coronary vessels are found between conditional *Klf4* knockout mice and control mice [7]. Because KLF4 has been shown to be expressed in both GATA4-negative and GATA4-positive cells in the heart [7], it is possible that the phenotype of smooth and cardiac muscle-selective *Klf4* knockout mice is caused by the *Klf4* deletion in cardiac cells except cardiomyocytes, such as cardiac fibroblasts. Further studies are needed to determine the expression pattern of KLF4 in more detail.

KLF4 inhibits atherosclerotic lesion formation

KLF4 is constitutively expressed in ECs [8], and it plays a key role in regulating vascular inflammation and thrombosis. Indeed, EC-specific deletion of the *Klf4* gene results in the increased atherosclerotic lesion formation in *Apoe* knockout mice when they are fed a high fat diet [19]. On the contrary, EC-specific over-expression of KLF4 decreases the atherosclerotic lesion formation in these mice [19]. Increased lesion formation in EC-specific *Klf4* knockout mice is accompanied by the augmented infiltration of CD45-positive inflammatory cells, as well as early thrombosis, as assessed by *in vivo* carotid injury assays and by *in vitro* fibrin clot formation assays [19]. Consistently, EC-specific *Klf4* knockout mice also results in the enhanced neointimal formation following vascular injury [20]. Although injury-induced down-regulation of SMC differentiation markers is unaffected by endothelial *Klf4* deletion, conditional *Klf4*-deficient mice exhibit the increased cellular proliferation rate and enhanced recruitment of inflammatory cells, such as macrophages and T-lymphocytes in injured arteries. Moreover, augmented induction of cell adhesion molecules, such as VCAM1 and E-selectin is observed in injured arteries of conditional *Klf4* knockout mice. Mechanistic studies demonstrate that KLF4 inhibits the inflammation-related activation of cell adhesion molecules by blocking the binding of NF-κB to the promoter region of these genes [20]. KLF4 physically associates with p65, a component of NF-κB [21]. By antagonizing NF-κB, KLF4 plays a protective role in vascular inflammation.

KLF4 in SMCs functions as a repressor of vascular proliferative diseases. Although KLF4 is not normally expressed in differentiated SMCs, it is induced in phenotypically modulated SMCs following vascular injury [9, 10]. Injury-induced expression of KLF4 transcriptionally activates the expression of $p21^{WAF1/Cip1}$ (CDKN1A), a cell cycle inhibitor, thereby repressing SMC proliferation [10]. KLF4-mediated activation of the *Cdkn1a* gene in SMCs is mediated by binding of KLF4 to proximal and distal KLF4 binding sites within the *Cdkn1a* promoter-enhancer. Of these, the distal KLF4 binding site is located within close proximity to the p53 binding site at the enhancer region of the *Cdkn1a* gene, and KLF4 and p53 cooperatively activate the transcription. As a result, deletion of the *Klf4* gene in mice results in the accelerated neointimal formation following vascular injury [10]. Recently, KLF4 has been shown to mediate the anti-proliferative effect of rapamycin in rat carotid arteries after balloon injury [22]. Rapamycin induces *Klf4* expression in cultured SMCs and in

carotid arteries *in vivo*, and knockdown of *Klf4* attenuates the anti-proliferative effect of rapamycin following injury. In addition, cyclosporine also induces *Klf4* expression in SMCs [23]. Moreover, KLF4 has been shown to regulate vascular calcification by cooperating with Runx2 [24]. As such, KLF4 is a potential target for the treatment of vascular proliferative diseases, such as atherosclerosis and restenosis after percutaneous coronary interventions.

Endothelial KLF4 modulates pulmonary arterial hypertension

Endothelial KLF4 has been shown to modulate pulmonary arterial hypertension [25]. Indeed, *VE-cadherin* (*Cdh5*) promoter-dependent endothelial *Klf4* deletion in mice results in the elevation of right ventricular and pulmonary arterial pressures, as well as right ventricular hypertrophy in response to chronic hypoxia. EC-selective *Klf4* depletion also causes the increased expression of endothelin-1 and the decreased expression of endothelial nitric-oxide synthase, endothelin receptor subtype B, and prostacyclin synthase in the lungs. Importantly, KLF4 expression is reduced in the lungs of patients with pulmonary arterial hypertension [25]. As such, KLF4 is likely to be a novel transcriptional modulator of pulmonary arterial hypertension.

KLF4 is required for the maintenance of normal endothelial function

In addition to preventing vascular diseases as described above, endothelial KLF4 also contributes to the maintenance of normal endothelial function. First, KLF4 mediates the beneficial effects of laminar flow, a major factor preventing vascular dysfunction and disease [26, 27]. Laminar flow-induced activation of the MEK5-Erk5 pathway elicits a vasoprotective phenotype via the induction of KLF4. Second, KLF4 is required for CDH5 expression [28]. In cultured human lung microvascular endothelial cells, siRNA-mediated knockdown of *KLF4* results in the decreased expression of CDH5, as well as the increase in endothelial barrier permeability. Moreover, siRNA-mediated knockdown of *Klf4* in mice augments lipopolysaccharide-induced lung injury and pulmonary edema [28]. Although it is possible that the phenotype observed in siRNA-treated mouse models is confounded by the effect of *Klf4* siRNA on non-ECs, these results suggest that KLF4 is required for the maintenance of endothelial barrier function. Further studies using EC-specific *Klf4* knockout animals are needed to determine if KLF4 functions cell-autonomously in ECs.

CONCLUSION AND PERSPECTIVES

Multiple lines of evidence indicate that KLF4 plays a protective role in the development and progression of cardiovascular diseases. KLF4 exerts multiple functions, including the anti-proliferative effect, the anti-inflammatory effect, the anti-thrombotic effect, and the anti-hypertrophic effect, by interacting with various proteins. Currently, 17 KLF family members are known, and some functions of KLF4 are redundant with those of other KLF family members, including KLF11 [29, 30] and KLF15 [31, 32]. It is of significant interest to determine whether KLF4 associates with these KLF family members to exert their overlapping functions. Moreover, it is interesting to determine which epigenetic factors, such as HDACs, histone methylases, and microRNAs, are required for the functions of KLF4. Because KLF4 has been shown to contribute to the reprogramming of multiple somatic cells into pluripotent stem cells [1-4], it is highly possible that alterations in epigenetic factors and mechanisms confer KLF4-dependent regulation of cardiovascular diseases. Further studies are needed to clarify the relationship between KLF4 and epigenetic factors in the cardiovascular system. Finally, it is hoped that further studies regarding the mechanisms and functions of KLF4 will contribute to the advancement of therapeutic and preventive approaches in multiple cardiovascular diseases.

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