Advances in the relationship between Kruppel-like factor 15 and cardiovascular disease research

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Kruppel-like factor 15 (KLF15) is a subtype of the Kruppel-like family of transcription factors (KLFs). KLFs have three high-fidelity zinc fingers at the carboxyl terminus that enable them to regulate the biological processes of proliferation, differentiation, cellular development, and apoptosis. KLF15 is highly expressed in the kidney, pancreas, and cardiac and skeletal muscle, and plays an essential role in the development and occurrence of multiple system diseases. In this paper, we underscored the important relationship between KLF15 and cardiovascular diseases such as atherosclerosis, heart failure, arrhythmia, aortic lesions, etc. On this basis, we identified KLF15 as a potential therapeutic target for the treatment of

cardiovascular disease. *Cardiovasc Endocrinol Metab* 7:37–41 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

Cardiovascular Endocrinology & Metabolism 2018, 7:37–41

Keywords: cardiovascular disease, Kruppel-like factor 15, zinc-finger family

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Received 15 May 2017 Accepted 10 November 2017

Background

Zinc fingers (ZnFs) proteins are involved in transcriptional processes, of which domains are first identified as a DNA-binding motif in transcription factor IIIA (TFIIIA) is pol III transcription factor specifically required for transcription of 5S rRNA genes. Recognition of extended DNA sequences by tandem ZnFs is considered to be largely determined by the specificity of short sequences by individual ZnFs. The typical C2H2-type ZnF (constitutes two cysteine and two histidine) is composed of an α -helix and an antiparallel β -structure. Four other amino acid residues are localized in specific positions of the N-terminal of the α -helix, which participate in DNA recognition by interacting with hydrogen donors and acceptors exposed on the DNA major groove. C2H2-type ZnFs are transcription regulators that play an important role in processes such as cellular development and differentiation, and malignant suppression [1]. In addition, C2H2-type ZnF of 'CACCC-element'-rich and 'GC-box'-rich binding motifs can recognize their specific target sites instead of nontarget sites, which plays a central role in structure stabilization and transcription regulation [2]. Kruppel-like factor 15 (KLF15) is a subclass of Kruppel-like family of transcription factors (KLFs). The characteristic structure of KLF15 is the three conserved C2H2-type ZnFs at the carboxyl terminal end that are responsible for specific binding with the DNA 'CACCC-element' and 'GC-box'; residues of cysteine and histidine in ZnF enable structure stabilization and transcription regulation. At the cellular level, ZnFs mediate and maintain KLF15 function. Recently, 18 KLFs have been discovered in placental mammals and named KLF1–KLF18 accordingly [3]. Different subtypes of

KLFs have similar regulatory domains, which enable them to interact in pathophysiological processes of disease. KLF15 was first identified as a repressor of the kidney-specific chloride channel CLC-K1. With in-depth research, KLF15 is expressed widely, but the highest expression levels are found in the kidney, liver, pancreas, and cardiac and skeletal muscle [4]. As a popular subtype of the KLFs, KLF15 has serine and proline on its amino terminus, which makes it different from other KLFs.

Cardiovascular disease is characterized by lipid metabolism dysfunction, endothelial cell injury, inflammation, and myocardial fibrosis. Although multiple therapies have been developed and are available, cardiovascular disease and its complications are the leading cause of morbidity and mortality worldwide. Hence, it is particularly important to determine the risk factors, diagnosis, and treatment of cardiovascular disease. This article primarily focused on the research progress of KLF15 on the pathophysiology of atherosclerosis (AS), myocardial remodeling, etc. Increasing understanding of novel molecular mechanisms highlights the direction of therapeutic targets for cardiovascular diseases.

Kruppel-like factor 15 and atherosclerosis

AS is a polyvascular disease that involves multiple intracellular macrophage formation, vascular smooth muscle cell (VSMC) migration and proliferation, connective tissue formation, and extracellular matrix deposition. AS is characterized by thickened intima, a hardened arterial wall, loss of elasticity, and narrowed momentous artery lumens. AS can lead to heart attack, stroke, or heart failure (HF) if left untreated.

Kruppel-like factor 15 and risk factors of atherosclerosis

The 'Lipid Infiltration Theory' points out that elevated plasma lipid is the central driver of the pathogenesis of AS. Low-density lipoprotein cholesterol enters into the tunica intima vasorum easily when endothelial dysfunction exists and then oxidizes into oxidized low-density lipoprotein cholesterol, which eventually makes the vessel more vulnerable to dangerous signals. Over the decade, a number of studies have shown that KLF15 is essential to maintain cardiac lipid metabolism. Mechanistically, KLF15 regulates myocardial lipid flux by the interaction with p300 and the recruitment of a critical coactivator to the promoters [5]. Also, KLF15 is essential for the ability of peroxisome proliferator-activated receptor α to induce target genes critical for cardiac lipid oxidation [6]. Hyperglycemia is another risk factor of AS, which is often accompanied by the strengthened function of coagulation factors and platelets. It is widely known that overexpression of KLF15 can increase the expression of the gene for phosphoenolpyruvate carboxykinase, a pivotal gluconeogenic enzyme [7]. Metformin inhibits hepatic glucose production by inhibiting the expression of phosphoenolpyruvate carboxykinase and G6Pase, and the effects of metformin on gluconeogenesis were inhibited by downregulation of KLF15 [8]. Both mechanisms have shown that KLF15 can promote gluconeogenesis and lead to an increase in blood glucose. A recent study showed that KLF15 formed a complex with LXR/RXR and repressed the expressions of sterol-regulatory element-binding protein-1c and its downstream lipogenic genes during early and euglycemic phases of food fasting. However, in the postprandial state, KLF15 has been suggested to be able to activate the downstream lipogenic enzyme expression [9]. This mechanism indicated that KLF15 reduced the risk of AS by maintaining the balance between glucose and lipid metabolism.

Kruppel-like factor 15 and vascular inflammation

To the best of our knowledge, vascular endothelial cell injury is a significant basis of AS. Endothelial cells can influence vascular remodeling by production of growth-promoting or growth-inhibiting substances and mediate inflammatory responses by expression of chemotactic and adhesion molecules, and release of chemokines and cytokines; they also regulate vascular smooth muscle cell contraction through the release of vasodilators and vasoconstrictors [10]. Endothelial dysfunction may contribute toward the development of a pathophysiological state of AS. In addition, VSMCs play a central role in maintaining blood vessel homeostasis. In response to injury, VSMCs proliferate, migrate, secrete extracellular matrix, and release cytokines/chemokines that can promote and increase inflammation [11]. KLF15, as an important regulator of VSMC proinflammatory activation, can alter

the acetylation status and activity of NF- κ B through direct interaction with histone [12].

Bone morphogenetic proteins are important regulators in vascular development and disease. Mediated by KLF15, endothelin-1 downregulates the bone morphogenetic proteins endothelial cell precursor-derived regulator in a dose-dependent manner, which is responsible for inhibiting endothelial cell inflammatory response and promoting angiogenesis in inflammatory stimuli of AS [13,14].

Kruppel-like factor 15 and reperfusion injury

Once the plaques of coronary artery are ruptured, a cascade of rapid thrombosis is activated to occlude the artery and can cause myocardial infarction. Ischemic reperfusion of the heart, a key therapeutic approach for acute myocardial infarction, may trigger severe reperfusion injury in the heart by inducing free radical production, early stress reaction, and cell apoptosis. Recently, an experiment using an ex-vivo model of ischemia-reperfusion proved that reperfusion injury could downregulate the expression of KLF15, but spironolactone could prevent reperfusion injury-induced decrease in KLF15 levels [15]. This study suggests that KLF15 is a positive and protective factor of the myocardium, and early application of mineralocorticoid receptor antagonists can prevent reperfusion injury to some extent.

Kruppel-like factor 15 and heart failure

HF is associated with increased risks of hospitalization and mortality, and therefore, it is a major health concern worldwide [16]. The main clinical manifestations of HF are pulmonary circulation congestion, systemic circulation congestion, and organ and tissue blood perfusion insufficiency, which involve complex pathophysiological processes including cardiac hypertrophy, ventricular remodeling, diastolic dysfunction, and changes in humoral factors. To date, several studies on the correlation between KLF15 and HF.

Kruppel-like factor 15 and cardiomyocyte hypertrophy

The sympathetic nervous and renin-angiotensin system stimuli activate cardiac hypertrophy, which is a harbinger of HF characterized by increased cell size or protein content, and reactivation of fetal genes [17]. During the development and pathogenesis of HF, cardiomyocytes undergo a series of changes such as pathological hypertrophy and cellular phenotype changes accompanied by re-induction of the fetal gene program regulated by human cardiac transcription factor-GATA4 [18]. KLF15 negatively regulates cardiac hypertrophy by suppressing the function of GATA4 and myocyte enhancer factor 2, and reducing the expression of atrial natriuretic factor and α -skeletal actin [18,19]. A previous work of Leenders *et al.* [19] showed that in the mouse model of angiotensin II-induced cardiac hypertrophy, overexpression of

KLF15 using recombinant adeno-associated viruses prevented the development of cardiac hypertrophy. Similarly, Fisch *et al.* [20] suggested that KLF15-null mice developed an eccentric form of cardiac hypertrophy in response to pressure overload, which could be alleviated by KLF15 overexpression. In contrast, loss of KLF15 contributed toward increased expression of atrial natriuretic factor, brain natriuretic peptide, and α -skeletal actin, and possibly other serum response factor-dependent target genes [21]. A recent research that studied the interaction of KLF15 with therapeutic agents has shown that decreased level of cardiac KLF15 in left ventricular hypertrophy was associated with chronic kidney disease and treatment of ACE inhibitors restored the level of Kruppel-like factor 15 and therefore reduced the severity of left ventricular hypertrophy [22].

Kruppel-like factor 15 and cardiac remodeling

HF is the final stage of various heart diseases, with multiple complex cellular and pathological processes. Ventricular remodeling is a vital pathophysiological process of heart failure, characterized by increased myocardial necrosis, cardiac fibroblast. The pathophysiological process will eventually cause severe ventricular ejection dysfunction. There has been significant progress in showing a role of connective tissue growth factor (CTGF) in the cardiomyocyte as a novel mediator of cardiac remodeling. In neonatal rat ventricular fibroblasts, overexpression of KLF15 inhibited basal and transforming growth factor- β 1-induced CTGF expression; in contrast, hearts from KLF15^{-/-} mice, in response to hemodynamic stress, showed elevated CTGF levels and increased myocardial collagen accumulation [23]. This study implied that KLF15 is a negative regulator of CTGF expression and cardiac fibrosis. Further investigations have shown that KLF15 can modulate the expressions of transforming growth factor- β , CTGF, and MRTF-A, and can ameliorate or even reverse myocardial fibrosis and improve cardiac function. The use of therapeutics that induce KLF15 in cardiac remodeling has been reported. A study has shown that resveratrol ameliorated cardiac remodeling in rats subjected to myocardial infarction by induction of KLF15 signaling [24]. Another mechanism of the role of KLF15 in controlling adult heart remodeling was through the regulation of β -catenin transcription, a critical regulator for cardiac progenitor cell differentiation [25]. This discovery may provide a tool for reactivation of this apparently dormant cardiac progenitor cell population in the adult heart, and can thus be considered an attractive approach to enhance endogenous cardiac repair.

Kruppel-like factor 15 and systolic dysfunction

Insufficient ATP supply to myocardial cells can decrease the calcium transport between sarcoplasmic reticulum and extracellular calcium transport, leading to systolic dysfunction, which is frequently accompanied by myocardial metabolic disorders. In addition, extensive remodeling of

the t-tubular network and associated excitation–contraction coupling proteins may contribute toward abnormal calcium handling in HF [26]. In previous studies, it has been shown that RYR2-mediated Ca^{2+} flux stimulates oxidative ATP production in diverse cell types [27]. Decreased RYR2 expression, structural organization, and function were associated with several models of metabolic cardiomyopathy, such as metabolic inflexibility in HF, cardiac hypertrophy, and ischemic cardiomyopathy [26]. Further work of Broun *et al.* [28] showed that application of a conditional gene knockout strategy (cRyr2KO mice) was sufficient to reduce mitochondrial Ca^{2+} and oxidative metabolism, activating marked metabolic reprogramming through Sirt1/Foxo1/Pgc1 α , Atf3, and Klf15 gene networks. Therefore, KLF15 may play a significant role in controlling systolic dysfunction.

Kruppel-like factor 15 and cardiac arrhythmia

Ventricular arrhythmia is a principal cause of sudden cardiac death, and has remained a major cause of death for decades. Modifications of repolarization, such as prolongation, shortening, and early repolarization, increase vulnerability to ventricular arrhythmias [29]. On the basis of the present study, it is clear that KChIP2, a gene encoding transient outward potassium current I, is present in nuclear protein fractions from cardiac tissue. KChIP2, which is essential in the maintenance of cardiac repolarization and depolarization, may be responsible for circadian rhythm and circadian rhythm numerous electrical remodeling events, ventricular arrhythmias, sudden cardiac death, etc. [30]. KLF15 can serve as a clock-dependent oscillator to control the circadian rhythmicity of cardiac ion-channel expression and myocardial repolarization by controlling the rhythmic expression of KChIP2. Either deficiency or excess of KLF15 can cause rhythmic QT variation loss, abnormal repolarization, and enhanced vulnerability in ventricular arrhythmias [31]. Furthermore, KLF15 governs a maximum ATP production phase, and remodeling and repair phases corresponding to the active and resting phases of a rodent [32]. Nevertheless, implanted telemetric devices recorded an electrocardiogram continuously for 5 days in conscious wild-type mice and KChIP2^{-/-} mice in light: dark periods and in complete darkness; similar diurnal (light: dark) and circadian (darkness) rhythms of RR intervals were found in WT and KChIP2^{-/-} mice. Hence, the contrasting results indicate that KChIP2 expression does not appear to underlie the circadian rhythm in the repolarization duration [33]. Previous studies have suggested that genes encoding potassium channels and accessory subunits were involved in susceptibility of atrial fibrillation (AF). However, in a total of 209 lone AF patients cohort study, no mutations in KLF15 were screened [34]. In this cohort KLF15 was not associated with lone AF.

Kruppel-like factor 15 and aortic lesions

The aorta is a large blood vessel in the human body, and its common lesions include aortic AS, aortic aneurysm,

and aortic valve disease. Aortic aneurysm may be complicated by acute aortic dissections, aneurismal rupture, and branch vessel occlusions, which is the most common cause of admission to the emergency department. In mouse models, deficiency of KLF15 could facilitate the processes of HF and aortic aneurysm, and the level of KLF15 is reduced in human aortic aneurysms. Hyperacetylation of p53 results in HF and aortic aneurysms by promoting apoptosis and inhibiting angiogenesis [35]. Deficiency of the KLF15 repressor inhibited p300-mediated acetylation of p53, and thereby reduced the activity of NF- κ B on inflammatory gene promoters [12]. In β -aminopropionitrile-induced rat AD models, the lentivirus was used for in-vitro and in-vivo KLF15 over-expression, which showed that the KLF15 mRNA was increased, whereas CTGFs and its target gene collagens I and III were downregulated [36]. These studies showed that KLF15 played an important role in aorta remodeling and aortic dissection formation.

Discussion

Despite the advances in the understanding of the molecular mechanisms of cardiovascular disease and the availability of cardiovascular drugs in the last several decades, cardiovascular diseases remain the leading cause of death and reduced quality of life worldwide [37]. This review found that KLF15 can inhibit myocardial remodeling, prevent HF and aortic aneurysm formation, regulate cardiac circadian rhythm, etc. It is noteworthy that the pathogenesis of cardiovascular disease involves a variety of cytokines or other factors; therefore, the definite mechanism of KLF15 in cardiovascular diseases is still unclear. With more in-depth studies, KLF15 may be a promising approach for the treatment of cardiovascular disease.

Acknowledgements

This work was supported by the Guangxi Natural Science Foundation (no. 2015GXNSFAA139207).

Conflicts of interest

There are no conflicts of interest.

References

- Razin SV, Borunova VV, Maksimenko OG, Kantidze OL. Cys2His2 zinc finger protein family: classification, functions, and major members. *Biochemistry (Mosc)* 2012; **77**:217–226.
- Liu L, Heermann DW. The interaction of DNA with multi-Cys2His2 zinc finger proteins. *J Phys Condens Matter* 2015; **27**:064107.
- Pei J, Grishin NV. A new family of predicted Kruppel-like factor genes and pseudogenes in placental mammals. *PLoS One* 2013; **8**:e81109.
- Uchida S, Tanaka Y, Ito H, Saitoh-Ohara F, Inazawa J, Yokoyama KK, et al. Transcriptional regulation of the CLC-K1 promoter by myc-associated zinc finger protein and kidney-enriched Kruppel-like factor, a novel zinc finger repressor. *Mol Cell Biol* 2000; **20**:7319–7331.
- Prosdocimo DA, Anand P, Liao X, Zhu H, Shelkay S, Artero-Calderon P, et al. Kruppel-like factor 15 is a critical regulator of cardiac lipid metabolism. *J Biol Chem* 2014; **289**:5914–5924.
- Prosdocimo DA, John JE, Zhang L, Efraim ES, Zhang R, Liao X, Jain MK, et al. KLF15 and PPAR α cooperate to regulate cardiomyocyte lipid gene expression and oxidation. *PPAR Res* 2015; **2015**:201625.
- Teshigawara K, Ogawa W, Mori T, Matsuki Y, Watanabe E, Hiramatsu R, et al. Role of Kruppel-like factor 15 in PEPCK gene expression in the liver. *Biochem Biophys Res Commun* 2005; **327**:920–926.
- Takashima M, Ogawa W, Hayashi K, Inoue H, Kinoshita S, Okamoto Y, et al. Role of KLF15 in regulation of hepatic gluconeogenesis and metformin action. *Diabetes* 2010; **59**:1608–1615.
- Takeuchi Y, Yahagi N, Aita Y, Murayama Y, Sawada Y, Piao X, et al. KLF15 enables rapid switching between lipogenesis and gluconeogenesis during fasting. *Cell Reports* 2016; **16**:2373–2386.
- Haga JH, Li YS, Chien S. Molecular basis of the effects of shear stress on vascular endothelial cells. *J Biomech* 2008; **41**:2331.
- Raines EW. PDGF and cardiovascular disease. *Cytokine Growth Factor Rev* 2004; **15**:237–254.
- Lu Y, Zhang L, Liao X, Sangwung P, Prosdocimo DA, Zhou G, et al. Kruppel-like factor 15 is critical for vascular inflammation. *J Clin Invest* 2013; **123**:4232–4241.
- Helbing T, Volkmar F, Goebel U, Heinke J, Diehl P, Pahl P, et al. Kruppel-like factor 15 regulates BMPER in endothelial cells. *Cardiovasc Res* 2010; **85**:551–559.
- Mouillesseaux KP, Wiley DS, Saunders LM, Wylie LA, Kushner EJ, Chong DC, et al. Notch regulates BMP responsiveness and lateral branching in vessel networks via SMAD6. *Nat Commun* 2016; **7**:13247.
- Mihalidou A, Ashton A. Os 15-04 mineralocorticoid receptor antagonists target endogenous repressor of hypertrophy. *J Hypertens* 2016; **34** (Suppl 1): e215–e216.
- Brown DA, Perry JB, Allen ME, Sabbah HN, Stauffer BL, Shaikh SR, et al. Expert consensus document: mitochondrial function as a therapeutic target in heart failure. *Nat Rev Cardiol* 2017; **14**:238–250.
- Hill JA, Olson EN. Cardiac plasticity. *N Engl J Med* 2008; **358**:1370–1380.
- Katanasaka Y, Suzuki H, Sunagawa Y, Hasegawa K, Morimoto T. Regulation of cardiac transcription factor GATA4 by post-translational modification in cardiomyocyte hypertrophy and heart failure. *Int Heart J* 2016; **57**:672–675.
- Leenders JJ, Wijnen WJ, Hiller M, van der Made I, Lentink V, van Leeuwen RE, et al. Regulation of cardiac gene expression by KLF15, a repressor of myocardin activity. *J Biol Chem* 2010; **285**:27449–27456.
- Fisch S, Gray S, Heymans S, Haldar SM, Wang B, Pfister O, Cui L, et al. Kruppel-like factor 15 is a regulator of cardiomyocyte hypertrophy. *Proc Natl Acad Sci USA* 2007; **104**:7074–7079.
- Leenders JJ, Wijnen WJ, van der Made I, Hiller M, Swinnen M, et al. Repression of cardiac hypertrophy by KLF15: underlying mechanisms and therapeutic implications. *PLoS One* 2012; **7**:e36754.
- Patel S, Velkoska E, Burrell L. Os 36-04 Kruppel like factor 15 is associated with cardiac hypertrophy in an experimental model of kidney disease. *J Hypertens* 2016; **34** (Suppl 1):e402–e403.
- Wang B, Haldar SM, Lu Y, Ibrahim OA, Fisch S, Gray S, et al. The Kruppel-like factor KLF15 inhibits connective tissue growth factor (CTGF) expression in cardiac fibroblasts. *J Mol Cell Cardiol* 2008; **45**:193–197.
- Rogers RG III, Otis JS. Antioxidant therapy attenuates post-infarct cardiac remodeling by driving expression of Kruppel-like factor 15. *FASEB J* 2016; **30**:3.
- Noack C, Zafiriou MP, Schaeffer HJ, Renger A, Pavlova E, Dietz R, et al. Kruppel-like factor 15 regulates Wnt/beta-catenin transcription and controls cardiac progenitor cell fate in the postnatal heart. *EMBO Mol Med* 2012; **4**:992–1007.
- Crossman DJ, Ruygrok PN, Soeller C, Cannell MB. Changes in the organization of excitation-contraction coupling structures in failing human heart. *PLoS One* 2011; **6**:e17901.
- Giacomello M, Drago I, Bortolozzi M, Scorsetto M, Gianelle A, Pizzo P, Pozzan T. Ca²⁺ hot spots on the mitochondrial surface are generated by Ca²⁺ mobilization from stores, but not by activation of store-operated Ca²⁺ channels. *Mol Cell* 2010; **38**:280–290.
- Bround MJ, Wambolt R, Luciani DS, Kulpa JE, Rodrigues B, Brownsey RW, et al. Cardiomyocyte ATP production, metabolic flexibility, and survival require calcium flux through cardiac ryanodine receptors in vivo. *J Biol Chem* 2013; **288**:18975–18986.
- Antzelevitch C. Role of spatial dispersion of repolarization in inherited and acquired sudden cardiac death syndromes. *Am J Physiol Heart Circ Physiol* 2007; **293**:H2024–H2038.
- Nassal DM, Wan X, Liu H, Deschênes I. Myocardial KChIP2 expression in guinea pig resolves an expanded electrophysiologic role. *PLoS One* 2016; **11**:e0146561.
- Jeyaraj D, Haldar SM, Wan X, McCauley MD, Ripberger JA, Hu K, et al. Circadian rhythms govern cardiac repolarization and arrhythmogenesis. *Nature* 2012; **483**:96–99.

- 32 Zhang L, Prosdocimo DA, Bai X, Fu C, Zhang R, Campbell F, *et al.* KLF15 establishes the landscape of diurnal expression in the heart. *Cell Rep* 2015; **13**:2368–2375.
- 33 Gottlieb LA, Lubberding A, Larsen AP, Thomsen MB. Circadian rhythm in QT interval is preserved in mice deficient of potassium channel interacting protein 2. *Chronobiol Int* 2017; **34**:45–56.
- 34 Nielsen MW, Olesen MS, Refsgaard L, Haunsø S, Svendsen JH. Screening of the i_{to} regulatory subunit KLF15 in patients with early-onset lone atrial fibrillation. *Front Genet* 2013; **4**:88.
- 35 Haldar SM, Lu Y, Jeyaraj D, Kawanami D, Cui Y, Eapen SJ, *et al.* KLF15 deficiency is a molecular link between heart failure and aortic aneurysm formation. *Sci Transl Med* 2010; **2**:26ra26.
- 36 Zhan B, Hu Z, Chen J, Zhu R, Zhao H, Yang J, *et al.* KLF15 overexpression protects β -aminopropionitrile-induced aortic rupture in rodent model via inhibiting connective tissue growth factor. *Thorac Cardiovasc Surg* 2017; **65**:120–125.
- 37 Steven S, Münzel T, Daiber A. Exploiting the pleiotropic antioxidant effects of established drugs in cardiovascular disease. *Int J Mol Sci* 2015; **16**:18185–18223.