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Review

The Involvement of Krüppel-like Factors in Cardiovascular Diseases

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Abstract: Krüppel-like Factors (KLFs) are a set of DNA-binding proteins belonging to a family of zinc-finger transcription factors, which have been associated with many biological processes related to activation or repression of genes, inducing cell growth, differentiation, death, and development and maintenance of tissues. In response to metabolic alterations caused by disease and stress, the heart will undergo cardiac remodeling, leading to cardiovascular diseases (CVD). KLFs are amongst transcriptional factors that take control of many physiological and, in this case, pathophysiological processes of CVD. KLFs seem to associate with congenital heart disease-linked syndromes, malformations because of autosomal diseases, mutations that relate to protein instability, and/or loss functions such as atheroprotective activities. Ischemic damage also relates to KLF dysregulation because of differentiation of cardiac myofibroblasts or a modified fatty acid oxidation, related to the formation of a dilated cardiomyopathy; myocardial infarctions, left ventricular hypertrophy and diabetic cardiomyopathies. MicroRNA have been involved in certain regulatory loops of KLFs as they may function as critical modulators of vascular smooth muscle cells in atherosclerosis, in heart failure and as markers of endothelial damage in acute myocardial infarction.

Keywords: Cardiovascular diseases; Krüppel -like factors; transcription factor regulation

1. Introduction

In response to metabolic alterations caused by disease and stress, the heart undergoes changes referred to as pathological remodeling, which involves hypertrophy and fibrosis, eventually leading to cardiac failure[1,2]. Similar metabolic changes can also affect the blood vasculature, which leads to structural alterations that potentially evolve into angiogenesis and atherosclerosis. These affections of heart and blood vessels are termed cardiovascular diseases (CVDs) [3–5].

Several comorbidities have been pathologically linked to CVD, including hypertension and diabetes [4,6–8], leading to an understanding of different factors involved in these diseases. One such family of factors is the Krüppel-like transcription factors (KLFs), which have recently acquired traction because of their involvement in various processes, including those regulating CVDs. KLFs are a set of DNA-binding proteins belonging to a family of zinc-finger transcription factors [9]. KLFs were named after their similarity to the *Drosophila melanogaster* Krüppel (from German, "crippled" protein), a member of the gap gene class involved in the thorax and anterior abdomen segmentation of *Drosophila* embryos [10], which, when presenting alterations, result in severe body abnormalities

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[11]. As mentioned earlier, KLFs are important gene transcription regulators capable of activating or repressing the expression of genes involved in several processes, including cell growth, differentiation, death, and the development and conservation of specialized tissues [12].

2. KLFs structure and domains

To date, researchers have identified 17 KLFs in the human genome, excluding the putative KLF-18 gene, which arises as a duplication of KLF17 [13]. KLFs are characterized by their three well-conserved carboxyl terminal C2H2 zinc finger domains, where two cysteines coordinate each zinc ion at one end of a β-sheet, and two histidines at the C-terminal α-helix, creating a tetrahedral structure which allows for folding: $\beta\beta\alpha$ protein configuration [14]. These zinc fingers are highly conserved throughout the family, sticking to the consensus sequence C-X2-5-C-X3-(F/Y)-X5-ψ-X2-H-X3-5-H, where X represents any amino acid and ψ is a hydrophobic residue [15]. A seven amino acid spacer TGEKP(Y/F)X can be found between each finger [16]. The first two zinc fingers have 25 amino acids each, while the third has only 23; at a particular level, each zinc finger can bound three base pairs in GC-rich regions, such as CACCC-, GC-, GT-box elements, located in promoters of target genes [17]. The amino-terminal domain is extremely varied arranged, containing binding domains capable of exerting repressive or activating functions. Finally, a nuclear localization signal (NLS) can be found near or within zinc fingers [18].

2.1. KLFs phylogenetic classification

As a consensus, KLFs subdivide into three phylogenetic groups because of their structural similarities and binding domains (Table 1).

Table 1. The consensus, according to the KLF group.

Group and members	Description	References
1. KLF -3 KLF -8 KLF -12	These mediate transcriptional repression by binding their C-terminal domain to the CtBP protein. CtBP can then mediate co-repression in an HDAC-dependant process, allowing histones to wrap DNA tightly. This mechanism was assessed by Turner and Crossley when they proved that mutations in the CtBP binding motif in KLF-3 failed to repress gene expression in SL2 cells. A gene repression HDAC-independent process could be executed by CtBP recruitment of PcG-associated proteins complex.	[19–22]
2. KLF-1 KLF-2 KLF-4 KLF-5 KLF-6 KLF-7	They mostly operate as transcriptional activators by recruiting acetyltransferase activity factors, such as CBP, p300, and P/CAF, promoting chromatin remodeling. Nevertheless, KLF-2 and KLF-4 also contain domains with repressor functions, continuous to the activation domains.	[23,24]
3. KLF-9 KLF-10 KLF-11 KLF-13 KLF-14 KLF-16	They have mostly been described as transcriptional repressors through their binding to SinA3. This interaction is possible because of a hydrophobic consensus sequence in these KLFs N-terminal domains, a conserved α-helical motif AA/VXXL that mediates their linking to SinA3 paired amphipathic helix domain, which then works as a scaffold for other chromatin modifiers, such as HDAC1, HDAC2, Mad, Ume6, MeCP2, N-CoR, and Ikaros.	[23,25]
No consensus groups. KLF-15 KLF-17 (-18)	These factors have not been incorporated into any of these phylogenetic groups since their interaction domains remain undetermined. Yet, tissue expression in bone, kidney, and testis has been reported.	[11,13,26]

KLFs as a family are found in several organ systems, namely the hematopoietic, gastrointestinal, respiratory, nervous, immune, and cardiovascular [27]. Hence, it is not uncommon for KLFs to have a ubiquitous expression pattern, such as in KLFs 6, 7, 8, 9, 10, and 11. Others have a more restricted expression, such as KLF-1, which is present in erythroid and mast cells, and KLF-2, which is involved in lung and vessel development [18,28]. Both KLF-1 and KLF-2 have an essential role in embryonic erythropoiesis since they can bind to genes involved in cell proliferation and cell cycle control, such as

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Forkhead Box M1 (FoxM1), Spinghosine kinase 1 (Sphk1), Parathyroid hormone 1 receptor (Pthr) and CD24a antigen, thus promoting the maturation of erythroid precursors [28].

Regarding the heart, KLFs expression and function continue at the initial elucidation stages. *KLF-15* expresses in cardiomyocytes and cardiac fibroblasts and upregulates postnatally, inhibiting cardiac hypertrophy by preventing myocardin (MYOCD) and serum response factor (SRF) interaction, thus, diminishing atrial natriuretic factor (*ANF*) and *α*-skeletal actin (*α-SKA*) expression [29,30]. Meanwhile, *KLF-13* has a marked reduced expression during the postnatal stage, moreover it also works as a cofactor with GATA4 and TBX5, an essential part of the transcriptional machinery required for inducing cardiac cell differentiation. Deletion of *KLF-13* (as well as other GATA4 modifier factors) has been associated with congenital heart defects, including Holt-Oram syndrome (discussed in a subsequent section), atrial and septal malformations, and ventricular hypotrabeculation [31].

3. Cardiovascular diseases (CVDs)

CVDs continue at the top of the list as causes of death worldwide [7,8]. Taking all into consideration, they account for nearly 56 million deaths every year [32]. CVDs affect both the blood vessels, as well as the heart at the mechanical, electrical, or cellular level, directly compromising nutrition and oxygenation, leading to damage and eventually death of the affected region [33–35]. At a fundamental level, we should note that the heart has little room for regeneration; therefore, damaged cardiomyocytes or other cardiac cells will eventually lead to cell loss, fibrosis, and heart failure [36–38]. The term CVDs is quite broad, encompassing a wide spectrum of diseases such as ischemic heart disease, heart failure, valvular heart disease, arrhythmias, high blood pressure, stroke, and others [6]. Yet, the most common causes of morbidity and mortality associated to CVDs are ischemic heart disease, stroke, and heart failure, which account for nearly 80% of all CVDs globally [35,39–41].

Interestingly, much information about the risk factors involved in developing CVDs are known. Many of these factors are preventable by behavioral changes. These preventable risk factors include tobacco consumption, physical inactivity, obesity, and unhealthy eating habits [42]. As an example of the latter, research has shown that a high lipid intake in the diet is associated with the development of atherosclerotic plaques, a condition directly related to CVDs [43,44]. Researchers have further linked high fat consumption and dietary obesity to an induced state of inflammation, generating adipose tissue and increasing the secretion of pro-inflammatory cytokines such as NF-kb, TNF-a, and INF-g [45]. This inflammatory state induces the production of reactive oxygen species (ROS) in the mitochondria, which leads to lipid peroxidation that can eventually induce several pathologies, including Alzheimer's and the development of aneurysms [46]. Excessive fatty acids lead to triglyceride and cholesterol esterification. Next, these lipids are taken-up by VLDL and later directed to LDL. In an already primed inflammatory state, high LDL levels can become oxidized (ox-LDL). Ox-LDL then becomes a problem as signals lead macrophages to engulf them, becoming foam cells that stack up in the arteries over time, eventually forming atherosclerotic plates [47]. Another preventable source of high ROS levels and oxidation is tobacco consumption. Particularly, vascular smooth muscle cells can react to external stimuli. Changes in these cells directly affect their differentiation from contractile cells to cells concerned with inflammation and ECM remodeling, reducing their expression of alpha smooth muscle actin (α -SMA) and smooth muscle 22 alpha (SM22α) and enhancing the production of inflammatory mediators as previously described with an outcome of atherosclerosis progression [48]. Unfortunately, not all risk factors are preventable, such as hyperglycemia related to type-1 diabetes, which has a genetic component, and its prevention is more complex. In this situation, a healthy lifestyle continues to be paramount [49]. At a molecular level, cardiac regulation and function involve a plethora of transcriptional factors that specify genes which take control of many physiological and, in this case, pathophysiological processes of CVDs[50]. One subset family of

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transcriptions factors involved in cardiac regulation during pathophysiological processes of CVDs is the KLF family.

3.1. KLFs in atherosclerosis

Atherosclerosis is a phenomenon characterized by the deposition of ox-LDL cholesterol in the arterial walls, is the primary pathology of CVD[47]. It develops from endothelial dysfunction, LDL retention, and the occupation of leukocytes in the subendothelial space, followed by signaling, recruitment, and differentiation of macrophages. Through the induction of nitric oxide synthase (eNOS and iNOS) and LDL oxidation, macrophages transform into foam cells, eventually forming atherosclerotic plates [51]. This change favors the evolution towards fibrous plaques that progressively reduce the diameter of the arterial lumen. Turbulent blood flow through a partially occluded vessel (or even normal fluctuations in the blood vessel path, such as in arterial bifurcations) damages the endothelium by shear force [51,52].

At the molecular level, *KLF-2* activation has been associated with laminar blood flow, a key protective force in the arterial walls that helps prevent atherosclerosis since it induces a protective phenotype in endothelial cells. In low-shear stress regions, KLF-2 inhibits a mechanosensory complex composed of platelet endothelial cell adhesion molecule (PECAM-1), vascular endothelial cadherin (VE-cadherin), and vascular endothelial growth factor receptor 2/3 (VEGFR2/3). These factors trigger the MEK2/ERK2 pathway to upregulate myocyte enhancer factor-2 (MEF2) and allow KLF-2 transcriptional activity. KLF-2 exerts as it downregulates vascular adhesion molecule-1 (VCAM-1) and E-selectin, molecules that support leukocyte migration and adhesion [53]. Researchers have identified suberanilohydroxamic acid as a potent pharmacological inducer of KLF-2, capable of repressing vascular inflammation and atherosclerosis [54]. Researchers believe that the main mechanism for this repression is the inhibition of thrombin-mediated cytokine as a repression mechanism of the protease-activated receptor 1 (PAR-1) [55].

According to Xie et al. (2021), one of the key changes in the progression of atherosclerosis is the transition of vascular smooth muscle cells (VSMCs) from a contractile phenotype to a proliferative phenotype. This transition leads to an increase in extracellular matrix secretion, resulting in the formation of arterial intima layer thickening. In this regard, *KLF-5* expression evidence seems to indicate an elevation in atherosclerotic plaques compared to normal human aortic tissue, suggesting that KLF-5 may play a role in promoting this phenotypic switch [56].

Transiently induced *KLF-4* after a vascular injury is not constitutively expressed in VSMCs [57]. In animal models, researchers found that after carotid artery ligation, KLF-4 activates rapidly in the SMC, which inhibited the expression of SMC differentiation marker genes (*SM-22* and *α-SMA*), as evidence suggests that KLF-4 blocks these markers through the binding of TGF-β's control element-containing promoter (5′-GAG-TGGGGCG-3′). In contrast, no binding of KLF-4 has been shown in intact carotid arteries[58–60]. Moreover, *KLF-4 KO* mice exhibited enhanced neointimal proliferation after vascular injury, contributing to the reduced arterial lumen. These results suggest that *KLF-4* is a negative regulator of neointima formation [61], also observed have been effects on non-vascular endothelial cells. KLF-4 does not affect SMC differentiation markers, but it downregulates TNF-*α*-induced VCAM1 expression by targeting and blocking the binding site of *NF-κB* to the VCAM1 promoter. Adhesion molecule expression promotes the accumulation of inflammatory cells that contribute to neointima formation [62].

Atherosclerosis and the shear stress forces associated with it lead to plaque rupture, causing thrombosis or vascular embolism, giving rise to ischemic heart disease in any of its two main clinical forms: angina or acute myocardial infarction (AMI) [63].

3.2. KLFs in ischemic disease, remodeling, and heart failure

Myocardial infarction (MI) is the most severe clinical manifestation of ischemic heart disease. It comprises the abrupt obstruction of blood flow in the main branches of the

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coronary arteries, eventually leading to cardiomyocyte ischemia [4,8,35]. In ischemic heart disease progression, fibrotic tissue replaces damaged muscle inducing geometric, biomechanical, and biochemical changes in the heart. This process is crucial to prevent ventricular wall rupture in the post-infarction period; however, an exaggerated fibrotic response has detrimental effects, leading to heart remodeling and a progressive loss of cardiovascular function to establish heart failure since it does not restore flood flow[64]. There is an affection of the myocardial tissue that leads to an overall decrease in oxygen or hypoxia, which ultimately causes necrosis of the area. Interestingly enough, as hypoxia and stress increases, there is an overall shift in signaling, which renders activation of a fetal program in the tissue, characteristic of development [65]. Elevation of embryonic signaling, as was seen in eight patients treated with pressure-controlled intermittent coronary sinus occlusion (PISCO), which resulted in the elevation of the transcription factors GATA4, MEF2C, TBX5, and HAND2 in blood samples. These are particular cardiac transcription factors, which have a history of being used in direct differentiation studies [66–68]. In the PISCO study, patient serums were collected and co-cultured with human fibroblasts and cardiomyocytes. Their findings indicated an upregulation in KLF-4; a known pluripotent stem cell inducer [57,69–71]. Unsurprisingly, KLF-4 promotes cardiac myofibroblast differentiation and collagen synthesis in angiotensin II-induced cardiac fibrosis through its binding to the *TGF-β1* promoter, activating the TGF-β1/Smad3 pathway, increasing the expression of a-smooth muscle actin, and the secretion of type I and type III collagen, contributing to the induction of a proliferative phenotype in cardiomyocytes [72].

Previously identified KLF-5 is a prohypertrophic factor that is increased in patients with terminal heart failure and mice with ischemic cardiomyopathy. The exact mechanisms by which KLF-5 induces cardiac hypertrophy remain unknown; however, research by Hoffman et al. confirmed that in mice subjected to left coronary artery ligation, *Klf-5* expression increased 2-fold at 24 hours and 4-fold at 2 and 4 weeks. A reduction in fractional shortening and expansion of the end-diastolic and systolic dimensions accompanied *Klf-5* upregulation. When using the pharmacological inhibitor of *Klf-5*, ML264, an improvement in echocardiographic parameters, such as ejection fraction, was observed, as well as a reduction in end-diastolic and systolic volume, exerting a protective effect against ischemic cardiomyopathy[73].

Previous research showed that KLF-5 could regulate *PPAR-α* expression and modify fatty acid oxidation (FAO). The heart depends on fatty acid oxidation (FAO) to produce ≈70% of its ATP and meet its energy demands [74]. This process transcriptionally depends on *PPAR-α*, which KLF-5 can activate via direct promoter binding [40,75]. Cardiac myocyte–specific ablation of KLF-5 consequently resulted in a decrease in *Ppar-α*, FAO, cardiac ATP levels, and triacylglycerol accumulation [40]. Interestingly, even though KLF-5 was being suppressed, the experimental model indicated signs of dilated myocardiopathy, such as a reduction of fractional shortening and an increase in left ventricle internal dimensions, showing that an excessive accumulation of lipids in the heart can indeed lead to dilated cardiomyopathy [40,75]. Although in this study, cardiomyopathy was suggested to develop in a KLF-independent manner, recent evidence shows a link between KLF-5 and ceramide biosynthesis. KLF-5 has been proposed as a direct transcriptional regulator of SPTLC1 and SPTLC2 (serine palmitoyltransferase [SPT] long-chain base subunit 1 and 2, respectively), enzymes involved in the rate-limiting step of ceramides de novo pathway synthesis, producing ceramides from serine and palmitoyl coenzyme A [73,75].

Regarding diabetic cardiomyopathy (DbCM), KLF-5 has been linked to oxidative stress via upregulation of NADPH oxidase 4 (NOX4) by directly binding to NADPH oxidase 4 promoter and inducing *NOX4* expression and leading to cardiomyocyte superoxide accumulation, mitochondrial abundance decrement and a change in the cardiac lipidome profile towards a ceramide-rich environment, therefore, contributing to DbCM physiopathology [75]. Meanwhile, dilated cardiomyopathy (DCM) is the most frequent cause of heart failure in young people. In the most severe cases, it is also a major reason for a heart transplant [76–78]. KLF-5 has been recently documented as being highly

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involved in the development of DCM. According to whole exome sequencing studies, *KLF-5* mutations were directly responsible for DCM with complete penetration within the proband's family members [77]. Hence, KLF-5 inhibition has been proposed as a strategy to treat heart failure and other cardiovascular diseases [75,79].

Genetic variations of *KLF-15*, documented as a hypertrophy inhibitor, have been studied in patients with type 2 diabetes. These findings have shown that a single nucleotide variation (SNV) in intron two of the *KLF-15* gene (rs9838915) was associated with increased left ventricle mass index and septal wall thickness. Additional follow-up of 5.6 years on average was performed, where 22 patients (7%) were hospitalized for the first time because of heart failure. In the latter, the adjusted risk of hospitalization for those patients with left ventricular hypertrophy (LVH) carrying the A allele was 5.5-fold greater than the G homozygous genotype. Therefore, the findings of this study propose the *KLF15* SNV rs9838915 A allele as a marker of left ventricle hypertrophy in patients with type 2 diabetes [26]. As a we have mentioned, the regulatory effects of KLF can play a role in CVD, the most important mechanisms can be seen on Table 2.

Table 2. KLFs involvement in different CVD, including the mechanism affected.

Disease	KLF involved	Effect	Mechanism	Reference
Atherosclerosis	KLF-5	Promoter	VSMCs proliferative phenotype switch via Myod repression.	[80]
	KLF-2	Protector	Reduces inflammation as it down-regulates VCAM1 and E-selectin.	[54,55]
	KLF-4	Protector	Inhibition of neointima formation via SM-22 and α-SMA repression.	[81,82]
Myocardial infarction	KLF-4	Promoter	Myofibroblasts differentiation and collagen secretion via TGF-β1/Smad3 pathway.	[72]
Left Ventricle Hypertrophy	KLF-15	Promoter	Rs9838915 associated with increased left ventricle mass index and septal wall thickness	[26]
Dilated cardiomyopathy	KLF-5	Promoter	Upregulation FOXO1	[29,75]
Diabetic cardiomyopathy	KLF-5	Promoter	Upregulation of NOX4, O–2 and ceramide accumulation	[75]

3.3. KLFs in Congenital Heart Diseases

While there is still much to be addressed in developmental biology, recent research has linked KLFs to birth defects, and in particular to certain congenital heart diseases. Out of these congenital heart diseases, the most prominently described disease linked to KLFs is the Holt-Oram Syndrome. The Holt-Oram Syndrome is an autosomal dominant disease characterized by upper-limb defects, congenital heart malformation, and cardiac electrical conduction related issues. Holt-Oram Syndrome has been typically associated with mutations in *TBX5*, even though new evidence has shown that KLF-13 plays a pathogenic role; as researchers have identified *KLF-13* as a genetic modifier for TBX5 [83,84]. During development, these two genes co-express in myocardium of the atrio-ventricular cushion, atrial septum, interventricular septum and ventricular trabeculae as early as E11.5 in the mouse embryo[84]. In silico sequence analysis has further revealed conservation of binding sites on the *Nppa* promoter for both *TBX5* and *KLF-13* genes, and other several key cardiac transcription factors such as *Nppb*, *Vegfa* and *Nos3*, all of them essential for heart development. To test the existence of a genetic interaction between these two transcription factors in heart morphogenesis, Darwich et al., (2017) created a *Tbx5* and *Klf13* double heterozygote mouse model, finding significantly lower left ventricular mass over body weight ratios and atrial septal defects in 80% of the mice. Gene expression patterns of heart development regulators (*Gata4*, *Mef2a*, *Erbb4*, *Vegfc*, and *Myh7*, among others) were further analyzed in physiologically normal *Klf13* or *Tbx5* heterozygotes, as well as the double

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heterozygous. Their results have indicated upregulation in *Tbx5* or *Klf13* heterozygotes, yet similar to control levels in the double heterozygote mice. These findings suggest a compensatory effect between the loss of either *Klf13* or *Tbx5*, but the inability to activate these compensatory pathways when the simultaneous decrease of both transcription factors [84].

Li et al., (2020) identified two KLF-13 variants in congenital heart disease (CHD) patients. They first identified a proline into serine transversion at amino acid position 163 (S156N) in a patient with tricuspid valve atresia, ventricular septal defects, and atrial septal defects. Followed by the transversion of serine into asparagine at position 156 (P163S) in a transposition of great arteries seven-month patient. Both S156N and P163S were found in the Nuclear Localization Signal 1 (NLS1) region, near to the KLF-13 DNA-binding domain. Expression of the S156N variant was noticeably higher than that of wild type and had increased transcriptional activity at activating the *BNP* promoter, suggesting S156N is a gain-of-function mutation. Otherwise, with P163S variant, demonstrated similar expression compared to wild type, yet lower transcriptional activity. Physical interaction with TBX5 was also assessed by co-immunoprecipitation. In this concern, P163S showed a decreased physical interaction with the TBX5 protein. In contrast, S156N had a significantly increased ability to interact with TBX5. Although, authors suggest that the overexpression of KLF-13 associated to S156N might be accompanied by higher protein instability, resulting in a loss-of function phenotype [85]

Lavallée et al., have previously described similar results, identifying *Klf-13* as a modifier of *Gata4*, a key transcription factor for the cardiac natriuretic peptide genes *Nppa* and *Nppb*. In this study, *Klf-13* Knockdown resulted in atrial septal defects, hypotrabeculation and hypoplastic myocardium in Xenopus embryos[31]. Mutations in *GATA4*, have been reported in patients with Fallot Tetralogy previously, although no direct relation between KLF-13 and this condition has been established yet[86].

Moreover, KLF-13 seems to interact physically and functionally with GATA-6, a transcription factor expressed in smooth muscle cells and cardiomyocytes [31]. Wang et al., (2020) reported a novel *KLF-13* loss-of-function variation, with reduced activation of *GATA-6*, *GATA-4* and *ANP* promoters. Researchers identified this mutation in a three generation Chinese family, in which 5 out of 18 living family members had double-outlet right ventricle and ventricular septal defects[87]. Other variations in KLF-13 have been linked to congenital heart defects, such as the Glu144*-mutant of KLF13 cannot trans-activate *VEGF-a* and *ANP* gene promoters, associated to patent ductus arteriosus and ventricular septal defect, as well as bicuspid aortic valve[88].

Recently, *KLF-4* has been linked to Marfan syndrome, a common inherited connective tissue disorder caused by mutations in *Fibrillin-1* gene, characterized for physical features such as increased height, scoliosis, arachnodactyly, lens dislocation, and cardiovascular disorders, including mitral valve prolapse and aortic aneurism that can trigger aortic dissection[89,90]. Using single-cell sequencing, Pedroza et al., identified *KLF-4* as one of several enriched expression genes in smooth muscle cells undergoing a phenotypic modulation towards fibroblasts, in aortic aneurysm tissue from a $Fbn1^{C1041G/+}$ Marfan syndrome murine model[91].

4. KLFs & miRNA in cardiovascular diseases

MicroRNA (miR)-145 is the most abundant miR in VSMC, overseeing the maintenance of cells in their contractile phenotype by promoting contractile genes[92]. The phenotype in which cells are is an important factor to take on account in the pathogenesis of atherosclerosis[93]. The VSMC phenotype increases atherosclerotic development because of its facility to migrate, proliferate and generate extracellular matrix proteins[94]. This phenotype switching is regulated by *KLF-4*, as suggested in several studies[80,95]. The overexpression of KLF-4 inhibits VSMC proliferation induced by PDGF [61,96]. miR-145 also has a role as a key regulator of *KLF-5*, *KLF-4*, and *MYOCD*, as it down regulates the first two genes by suppressing their transcription (which are repressors of MYOCD) and

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directly stimulates the translation of myocardin[92]. Researchers have found that miR-145 expression (whose one of his target genes is *KLF-5*) was found to be considerably higher in the normal aortic samples group, accompanied by a higher expression of contractile proteins such as calponin and α-SMA, compared to the atherosclerotic group where circulating levels of miR-145 were reduced [80,97]. In animal models of vascular diseases, miR-143/miR-145 were found to be downregulated, albeit this has not been confirmed in humans. Current research further proposes that miR-145 or miR-143 are part of the regulatory loop for KLF-4, KLF-5, MYOCD, and SRF; critical transcription factors development of SMC phenotype, and lacking SMC correct differentiation, could lead more easily to develop atherosclerosis[98,99]. *KLF-5* inhibition via miR-145 results in failure to repress MYOCD, a transcriptional cofactor for SRF, which commands the expression of multiple smooth and cardiac muscle-specific genes, such *SM-22*, *ANP*, *MLC-2V* and *α-MHC* [97,100,101]. Transient decease in miR-145 expression 3 days post myocardial infarction was associated with an increase in *KLF-5* and a decrease in *MYOCD*; in addition, miR-145 is necessary for myocardin-induced cell reprograming of adult fibroblast into SMC and to induce differentiation of multipotent neural crest stem cells into VSMC [102]. These data suggest that the miR-145/ KLF-5 / MYOCD path might be a critical modulator of VSMC in atherosclerosis.

A study using human coronary artery smooth muscle cells (HCASMCs) cultured under hyperglycemic conditions found that the repression of miR-145 resulted in *KLF-4* upregulation and thus, a decrease in MYOCD expression. This response mediated by Ang II secretion in HCASMCs, resulted as a reaction to high glucose conditions, which developed in facilitating migration of VSMC, as well as reducing the expression of VSMC differentiation marker genes, such as *α-SMA*, transgelin, and smoothelin, among others [95].

MiR-133 has also been associated with VSMC phenotypic modulation. miR-133 is capable of downregulating *KLF-4* via suppression of its coactivator transcription factor, Sp1. In this process, miR-133 targets Sp-1, preventing KLF-4 activation and making it unable to displace MYOCD from the SRF complex, determining the upregulation of smooth muscle genes, like *MYH11* [103].

Horie et al., assessed the role of miR-133 in chronic heart failure, identifying *KLF-15* as another miR-133 target. In their study, miR-133 was shown to reduce KLF-15 and GLUT4 protein expression[104]. KLF-15 and MEF2A synergistically bind to the GLUT4 promoter, therefore increasing glucose uptake in cardiomyocytes, a process of vital importance for the maintenance of myocardial energetic supply[105,106]. These results suggest miR-133 may play a role in the perturbed energetics of heart failure.

In another study, rats were infarcted to assess the role of miR-92a and its relation to *Klf-2* and *Klf-4* in endothelial injury after left coronary artery ligation. Herein, this study demonstrated that in animal models, endothelial injury markers *H-Fabp*, *vWF*, and miR-92a were significantly higher than the control group, while vasoprotective factors *Klf-2* and *Klf-4* were downregulated through miR-92a binding to their 3′ UTR. The suppression of miR-92a seems to promote endothelial activation, cardiac cell proliferation, and the decrease of apoptosis after AMI, proving that both *Klf-2* and *Klf-4* are involved in the protection and modulation of endothelial cells [107]. Similar results were obtained when using of antimiR-92a, in addition, decreased macrophage and T lymphocyte accumulation as well as, a marked reduction in atherosclerosis (32%, as compared to the non-treated group)[108].

miR-32-5p targets the expression of *KLF-2.* In a recent study, researchers found elevated serum levels of miR-32-5p in patients with AMI, and reduced expression of KLF-2 [109]. KLF-2 possesses atheroprotective properties [107], hence having an adequate expression of this gene could prevent the development of a cardiovascular disease. In another study, miR-363-3p was upregulated in serum of AMI patients, showing that the expression of this miR was positively correlated with the concentration of endothelial injury biomarkers. As confirmed in rat studies with knockdown of miR-363-3p, which showed that endothelial injury biomarkers are reduced. In the same study, they observed that the

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activity of KLF-2 was inhibited with the upregulation of miR-363-3p, leading patients towards a higher probability of suffering AMI [110].

Regarding ischemic damage, miR-125b-5p was linked to a cardioprotective effect in the onset of AMI. Using several prediction algorithms, researchers have identified proapoptotic *BAX1* and *KLF-13* as miR-125b-5p targets. In this study, this research group showed that *in vivo* repression of miR-125b-5p associates to a higher mortality after left coronary artery ligation, left ventricular disfunction, enhanced susceptibility to cardiac rupture, higher levels of *ANP* and *TNF-α*, and larger fibrotic regions. In vitro analysis showed that miR-125b-5p could induce an increase in p-AKT levels, suggesting a function as a pro-survival miR in cardiomyocytes. Furthermore, researcher proved that β-blocker carvedilol was capable of upregulating miR-125b-5p (process accompanied by a decrease in BAX1 and KLF-13) [111]. These findings suggest miR-125b-5p as a carvedilol-responsive miR, mediator of improved cardiac function after AMI, via blocking of pro-apoptotic proteins.

miR-let-7g demonstrated an increase in the expression of α-SMA and calponin by downregulation of PDGF-β leading to a reduce interaction of KLF-4 and SRF which derepressed MYOCD; this maintains VSMC contractile phenotype and therefore reduced formation of atherosclerotic plaques [112]. There have been some miRNAs related with the AMI but not associated with KLF signaling. miR-139-5p has been involved in regulating cardiomyocyte proliferation and apoptosis. Finally, further research has also confirmed that miR-139-5p increases in the serum of AMI patients [113].

Table 3. miRNAs involvement in KLF regulation during CVDS.

MiRNAs	Cardiovascular diseases	Target or signaling pathways	Level of expression	Ref.
miR-143/145	Pro-atheroerotic	KLF4/5	• ↓expression	[114]
miR-1		KLF4	• MiR-1 induces SMC differentiation through the repression of Klf4	[115]
miR-137-3p		↓KLF15	• Promote ischemia	[116]
miR126		KLF2	• ↑expression of miR-126 ↑KLF2 activated VEGF	[117]
miR29a		KLF15	• ↑ expression of miR-29a ↑miR29 increased KLF15 stability by Fbw7/CDC4.	[118]
miR-410	Anti- atherosclerosis	KLF5	• HDAC1 • KLF5 promote IKB alpha ↓NFKB	[119]
mmu-miR-107, mmu-miR-142-5p, mmu-miR-143, mmu-miR-155		KLF2	• FOXO1 regulates the expression of the downstream transcription factor KLF2 in endothelial cells	[120]
miR-10a	Myocardial infarction	KLF4	• miR-10a rejuvenated aged hBM-MSCs which improved angiogenesis and cardiac function in injured mouse hearts.	[121]
miR-27a	Myocardial infarction	KLF5	• miR-27a expression could be transcriptionally suppressed by KLF5 and inactivated the TGF-β/Smad2/3 signaling pathway	[122]
mIR-363-3p	AMI	KLF2	• ↓miR-363-3p reduces the concentration of endothelial biomarkers and promotes the vascular endothelial cell proliferation, and this protective effect on endothelial injury may be exerted by targeting KLF2.	[110]

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miR32-5p	AMI	KLF2	• miR-32-5p promotes endothelial cell viability by KLF2	[109]
miR-125b-5p	AMI	KLF13	• miR-125b-5p protects the heart against AMI by blunting CM death in response to injury in part through its repression of bak1 and klf13	[111]
miR-150	AMI	KLF13	• Increasing KLF13 expression via ↓ miR-150.	[123]
mIR-92a	AMI	KLF2 KLF4	• miR-92a promoted endothelial activation, cardiac cell proliferation and apoptosis decrease after AMI, proving that both KLF-2 and KLF-4 are involved in the protection and modulation of endothelial cells	[124]
miR-124		KLF6 and STAT3	• downregulation of miR-124 and Sp1 levels was increased sharply in human aortic media from clinical specimens of aortic dissection	[125,126]
miR-let-7g	Atherosclerosis	KLF4, SRF, α-SMA, calponin, PDGF-B	• Increasing ↑ α-SMA expression via ↓ KL4 y SRF which depresses to Myod.	[112]
miR-139-5p	AMI	Serum AMI	• Increases ↑ serum of AMI patients.	[113]

5.Conclusion

The extensive KLFs family has been associated with many biological processes related to cell growth, differentiation, death, and development and maintenance of tissues in many eukaryotic organisms. In cardiovascular system dysregulation, KLFs seems to be associated with CVDs such as a) CHD-linked syndromes or malformations because of autosomal diseases related to instability and/or loss of function, b) loss of atheroprotective activities c) ischemic damages due to differentiation of cardiac myofibroblasts or a modified fatty acid oxidation as related to the formation of a dilated cardiomyopathy, d) cardiovascular complications such as myocardial infarctions, left ventricular hypertrophy and diabetic cardiomyopathies. Finally, several miR have been linked to AMI but not all are related to KLFs signaling. Others miR have been involved in certain regulatory loops of KLFs as they may act as critical modulators of VSMC in atherosclerosis, in abnormalities of heart failure and as markers of endothelial damage in the AMI.

Author Contributions: Conceptualization: MGS-S, JDM-M, GRP-R, Investigation: GRP-R, Resources: LG-O, GA-M, Writing-original draft preparation: AGQ-R, DFB-C, ENG-T, JAR-P, Writing-review editing: MGS-S, JLD-G, JFI, Supervision JFI

Funding: "This research was funded by CONACYT, CF-51208"

Institutional Review Board Statement: "Not applicable"

Informed Consent Statement: "Not applicable."

Data Availability Statement: "Not applicable"

Acknowledgments: The authors are grateful for the support provided by Sergio Lozano-Rodríguez (Scientific Publications Support Coordinator, UANL) in reviewing this manuscript

Conflicts of Interest: state "The authors declare no conflict of interest."

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