MicroRNAs and Cardiovascular Disease

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Published online: 18 January 2013
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Abstract MicroRNAs (miRNAs) regulate gene expression by binding to their targets and promoting RNA degradation and/or inhibiting protein translation. In recent years, miRNAs have revolutionized our understanding of gene regulatory networks, providing new prospective tools to manage disease. Atherosclerosis and other cardiovascular diseases are a leading cause of disability and death in the US and in other western populations and pose an enormous burden on our healthcare system. Altered lipid homeostasis in liver or in the artery wall, and disruption of endothelial and smooth muscle cell function have been shown to contribute to the onset and progression of cardiovascular disease. This review focuses on recent advances in the field of vascular biology- and lipid metabolism-related miRNomics.

Keywords miRNA · Lipid metabolism · Gene expression · Cardiovascular disease

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Introduction

MicroRNAs (miRNAs) are non-coding, ~22 nucleotide RNAs that bind to specific, partially complementary sequences of target mRNAs, generally in the 3'-UTR [1–4]. This leads to RNA interference and/or translational repression of the target gene, thus resulting in reduced expression of the target mRNA [1]. miRNAs can be transcribed from their own promoter or can be encoded within the introns of other genes. It is thought that in the latter case these miRNAs are expressed when the “hosting” mRNA is transcribed [1–4]. Regardless, the original Pri-miRNA (up to several kb) is first processed by the exonuclease Drosha, and the resulting Pre-miRNA (100–150 nt) is then exported to the cytoplasm for further cleavage by Dicer to generate the mature miRNA (20–24 nt), which is finally incorporated into the RNA-induced silencing complex. Other, non-canonical pathways that are independent on Drosha or Dicer have been also described. Expression of particular miRNAs has been found in tissue, developmental and even disease-specific, revolutionizing our understanding of gene expression regulatory networks. Hence, recent studies have shown that miRNAs function as key mediators in multiple physiological and pathological-related biological processes. In this review we focus on the role of miRNAs in cardiovascular disease.

miRNAs, as Regulators of Endothelial Cell (EC) Function

Vascular ECs line the entire circulatory system and form the barrier between blood and all tissues. ECs are involved in many aspects of vascular biology ranging from inflammation, vasodilation and angiogenesis [5, 6]. Impaired EC function results in hypertension, thrombosis, inflammation and atherosclerosis [7]. Since 2006, several reports have

demonstrated the critical role of miRNAs in regulating almost all ECs functions [8•, 9•, 10•, 11]. Here, we summarize the recent findings in the field highlighting the contribution of miR-126, miR-210, and the miR-17-92 miRNA cluster in controlling EC activation.

miR-126

One of the first EC miRNA identified was miR-126, an EC-enriched miRNA that controls vascular inflammation by inhibiting the adhesion molecule VCAM-1 [12]. miR-126 also plays an important role in regulating angiogenic signaling and vascular integrity by directly repressing negative regulators of the vascular endothelial growth factor (VEGF) signaling pathway including the Sprouty-related protein (SPRED) 1 and the phosphoinositide-3 kinase regulatory subunit 2 (PIK3R2/p85 β) [13, 14]. In addition to the gene regulatory role of miR-126 in the cell where it is generated, several studies have shown that miR-126 is secreted in microparticles and apoptotic bodies and delivers paracrine signals to recipient vascular cell [15]. In this regard, Weber and colleagues showed that EC-derived apoptotic bodies were generated during atherosclerosis and demonstrated a paracrine signal to recipient vascular cells that triggered the production of CXCL12 [15]. Interestingly the administration of apoptotic bodies or miR-126 limited atherosclerosis by promoting the incorporation of Sca-1+ progenitor cells in the vessel wall. miR-126 has been also found in plasma vesicles and its expression is significantly reduced in plasma of patients with type-2 diabetes [16].

In addition to miR-126, other miRNAs including miR-181b, regulate EC activation by negatively modulating NF- κ B signaling pathway [17]. miR-181b expression is regulated by tumor necrosis factor (TNF) α and targets importing 3a (KPNA4), a protein required for NF κ B nuclear translocation. Similarly, TNF α also increases the expression of miR-17-3p and miR-31, which target ICAM-1 and E-selectin, thereby reducing inflammation [18].

miR-210

Hypoxia occurs during several physio-pathological circumstances including myocardial infarction, ischemia and tumor growth [19]. One of the early responses to hypoxia is the activation of ECs, which then migrate and proliferate in order to generate new vessels. Several reports recently demonstrated that the hypoxia-induced miR-210 regulates multiple aspects of the cellular response to low oxygen exposure including angiogenesis, migration, proliferation and metabolism. Thus, miR-210 regulates angiogenesis by controlling the expression of receptor tyrosine kinase Ephrin-A3 (EFNA3) [20, 21]. miR-210 overexpression also enhances endothelial cord formation, suggesting that

miR-210 alone is sufficient to promote angiogenesis [20]. Interestingly, the expression levels of miR-210 correlates with VEGF levels in breast cancer patients [22].

miR-17-92

miR-17-92 is a polycistronic microRNA cluster that includes seven mature miRNAs (*miR-17-5p* and *-3p*, *miR-18a*, *miR19a* and *b*, *miR-20a* and *miR-92a*). This cluster was originally called oncomiR-1 for its strong association with a variety of tumors [23]. Several reports highlighted the contribution of several members of the cluster in regulating angiogenesis [11, 24–26]. Suárez and colleagues demonstrated that angiogenic chemokines such as VEGF induced the expression of some components of the miR-17-92 cluster, leading to the repression of the thrombospondin-1, an anti-angiogenic molecule [11]. Other studies showed the important role of specific members of the cluster in regulating postnatal neovascularization [24–26]. In this regard, miR-92a regulates angiogenesis both in vitro and in vivo [25]. Thus, overexpression of miR-92a impairs angiogenesis in vitro and intersomic vessel growth in zebrafish [25]. Most importantly, anti-miR-92 therapy improves functional recovery after acute myocardial infarction [25]. Mechanistically, miR-92a directly regulates the expression of integrin subunit α 5, a crucial regulator of EC growth. Moreover, miR-92a also regulates the endothelial nitric oxide synthase (eNOS), an enzyme that manufactures nitric oxide (NO) in ECs, thereby regulating vascular tone and angiogenesis. The molecular mechanism by which miR-92a regulates eNOS expression is complex. It has been recently shown that atheroprotective laminar flow reduces miR-92a levels, which inhibits KLF-2, a transcription factor that regulates eNOS expression [24]. KLF-2 also increases the expression of miR-143/145 in ECs [27]. Interestingly, both miRNAs are secreted in microvesicles and delivered to vascular smooth muscle cells (VSMCs) where they induce an atheroprotective effect by inhibiting the expression of the ETS oncogene family (ELK1), KLF4, calcium/calmodulin-dependent protein kinase II delta (CAMK2d), slingshot homolog 2 (SSH2), phosphatase, actin regulator 4 (PHACTR4) and cofilin 1 (CFL1) [27].

miRNAs VSMCs and Restenosis

VSMCs are the most abundant cells in the large arteries and play key roles in regulating vascular tone. These specialized cells secrete extracellular matrix components such as collagen and elastin, which determine the mechanical properties of the arteries. Alterations in VSMC function results in multiple cardiovascular diseases including hypertension, aortic aneurism, Marfan syndrome, supravulvar aortic stenosis and

Williams–Beuren syndrome (WBS), which is characterized for mutations in the *elastin* gene [28–31]. Importantly, miRNAs play important roles in regulating VSMC functions and several studies have elegantly shown their potential for developing new drugs for ameliorating cardiovascular disorders including elastin haploinsufficient disorders [32].

The initial evidence that demonstrated the importance of miRNAs in controlling VSMC functions were provided using a mouse model with a conditional deletion of *Dicer* in VSMC [33, 34]. Absence of *Dicer* in VSMC results in embryonic lethality at E16.5 associated with extensive hemorrhage, defective organization of the elastic lamina and loss of contractile function in the umbilical artery [33]. In a following study, the same authors showed that miRNAs were also important in regulating VSMC homeostasis post-natally by deleting *Dicer* using a conditional inducible mouse model [34]. Importantly, the absence of *Dicer* leads to a dramatic reduction in blood pressure and to decreased contractile function in the bladder. Altogether, these studies suggest that the *Dicer*-dependent miRNAs are critical for the developmental and postnatal regulation of VSMCs function. In addition to the global role of miRNAs, specific miRNAs including miR-21, miR-143/145 and miR-29 were shown to play important roles in controlling VSMC functions [35–40, 41•, 42, 43•, 44, 45•, 46–48•, 49, 50•, 51].

miR-21

miR-21 regulates VSMCs proliferation and survival by inhibiting the expression of the phosphatase and tensin homolog (PTEN) and increasing Bcl-2 respectively [38]. Both effects were demonstrated in a carotid injury model. In addition to its role in cellular growth, miR-21 also promotes VSMCs differentiation in response to transforming growth factor and bone morphogenetic factor-4 [39]. Several reports have recently demonstrated the important role of miR-21 in regulating several cardiovascular disorders including the development of abdominal aortic aneurysm (AAA) and cardiac fibrosis [40]. miR-21 expression increases during AAA progression and its overexpression induces VSMCs proliferation and decreases apoptosis in the vascular wall [40]. Conversely, miR-21 inhibition diminished its pro-proliferative effect, leading to a significant increase in the size of AAA [40].

miR-143/145

The importance of the *miR-143/145* miRNA cluster in controlling VSMCs functions was initially demonstrated in the *miR-143/145* double mutant mice [41•, 42, 43•]. These animals are viable but they show a significant reduction in blood pressure and their response to vascular injury is significantly diminished [41•, 42, 43•]. Mechanistically,

miR-143/145 deficient-VSMCs show a marked inhibition of their migratory capacity accompanied by important alterations in the actin stress fibers. miR-143/145 also regulates VSMC plasticity and their absence results in a synthetic phenotype and lost of their contractile abilities [43•, 45•, 47]. These data demonstrate that miR-143/145 plays an important role in modulating VSMC phenotypic properties, which is critical during the progression of several vascular diseases such as atherosclerosis and AAA [41•, 46].

miR-29

miR-29 family members are encoded in two different loci that give rise a bicystronic precursors, which include *miR-29a/b1* and *miR-29b2/c*. Several groups have published data recently highlighting the important role of miR-29 in controlling the expression of many components of the extracellular matrix such as elastin and collagen [48•, 49, 50•, 51, 52]. miR-29 is upregulated in aged arteries and in several murine models of aortic aneurysms [48•]. Very interestingly, therapeutic inhibition of miR-29 increases the expression of elastin and collagen and impairs the progression of aortic dilatation after angiotensin-II treatment [48•]. Similarly, miR-29 antisense oligonucleotides (ASO) increase the expression of elastin gene in cells isolated from WBS patients [50•]. These observations suggest that antagonism miR-29 in vivo might be a useful approach for treating elastin haploinsufficiencies and cardiovascular disease characterized by media destruction including AAA and atherosclerotic.

miRNAs, Lipid Metabolism and Atherosclerosis

Atherosclerosis is a progressive disorder of the vascular wall wherein massive amounts of cells and lipids accumulate in the sub-endothelial space [53]. Advanced plaques can eventually rupture leading to thrombotic events that are the primary cause of heart attack, stroke, and peripheral artery disease, which collectively account for >30 % of all deaths in the US. Multiple studies have recognized abnormal cholesterol homeostasis as a risk factor for the development of atherosclerosis. In the past few years, several laboratories have shed light on how miRNAs regulate lipid homeostasis (Fig. 1) and pointed to the potential of targeting specific miRNAs as potential new therapeutic weapons to manage atherosclerosis.

miR-33a/b

In 2010, five publications simultaneously reported that *miR-33* (also known as *miR-33a* or *miR-33a-5p*) is expressed from within an intron of *Srebp2*, is induced by low intracellular

Fig. 1 miRNA regulation of lipid metabolism. Schematic overview of the most relevant miRNAs involved in regulating lipid metabolism in the liver and adipose tissue. *Question mark* indicates indirect target gene

cholesterol or following treatment with statins, and represses the expression of the sterol transporters ABCA1 and rodent, but not human, ABCG1 [54•, 55•, 56•, 57, 58•]. In agreement with these data, ASOs that silence miR-33 result in increased circulating high-density lipoprotein cholesterol (HDL-C) in mice [54•, 55•, 56•]. Primates, but not rodents, express a second *miR-33* gene (*miR-33b* or *miR-33b-5p*) from an intron of *Srebp1*. Importantly, both *Srebp* genes are differentially regulated by dietary challenges or statin treatment. Hence, humanized mice in which transgenic *miR-33b* is engineered to be expressed from an intron of *Srebp1* will be necessary to study the role of miR-33b on lipid metabolism. Initial studies also showed that miR-33 targeted genes involved in fatty acid β -oxidation, such as carnitine palmitoyltransferase 1A (*Cpt1a*), carnitine O-octanoyl transferase (*Crot*), and hydroxyacyl-CoA dehydrogenase-3-ketoacyl-CoA thiolase-enoyl-CoA hydratase (trifunctional protein) β -subunit (*Hadhb*) [57, 59•]. Finally, a recent report showed that miR-33 also targets the hepatic bile transporters ABCB11 and ATP8B1 [60•]. Hence, silencing hepatic miR-33 expression resulted in a concomitant increase in both circulating HDL-C and bile secretion in mice.

Treatment of mice with anti-miR-33 oligonucleotides led to increased reverse cholesterol transport (RCT) in vivo

[60•, 61]. The RCT pathway mobilizes peripheral cholesterol into HDL back to the liver, where both cholesterol and its metabolite bile acids are secreted into bile for final excretion through the feces. In this context, the RCT is regarded as atheroprotective and strategies that promote/accelerate the flow of cholesterol through this pathway are predicted to be effective in reducing the risk of cardiovascular disease in patients. Collectively, the initial reports on miR-33 suggested that patients with hypercholesterolemia might benefit from a therapy that incorporated silencing of miR-33 expression (e.g. miR-33 ASOs). In agreement with this notion, recent studies in non-human primates showed that treatment with miR-33 ASOs for 16 weeks increased circulating HDL-C, while at the same time reduced very-low density lipoprotein (VLDL)-triglycerides [62•]. These latter authors also reported that a 4-week antisense treatment of atherosclerosis-prone *Ldlr* mice with pre-existing atherosclerotic plaques resulted in accelerated regression of the atheromata [61]. Similarly, complete absence of miR-33 significantly reduces the progression of atherosclerosis in *Apoe*-deficient mice [63]. Based on these studies, miR-33 has emerged as a promising therapeutic target to manage cardiovascular disease. A more recent study, however, indicated that anti-miR-33

Cell type	microRNA	miR-target	Function
ENDOTHELIAL CELL	<i>miR-126</i>	VCAM-1 SPRED1, PIK3R2/p85 β	Leukocyte adhesion Vascular integrity
	<i>miR-181b</i>	KPNA4	Leukocyte adhesion
	<i>miR-17-3p</i>	ICAM-1, ESEL	Leukocyte adhesion
	<i>miR-31</i>	ICAM-1, ESEL	Leukocyte adhesion
	<i>miR-210</i>	EFNA3	Angiogenesis
	<i>miR-92a</i>	ITGA5, eNOS?	Angiogenesis
VASCULAR SMOOTH MUSCLE CELL	<i>miR-21</i>	PTEN	Growth and survival
	<i>miR-143/145</i>	PDCD4	Contractile function
		KLF5, KLF4, ELK1	VSMC differentiation
	<i>miR-29</i>	ELN, COL1A1, COL3A1	Vascular integrity
MACROPHAGE	<i>miR-33a/b</i>	ABCA1, ABCG1 NPC1 CROT, CPT1a, HADHB, AMPK α 1 RIP140	Cholesterol efflux Cholesterol transport Fatty acid oxidation Inflammation
	<i>miR-758</i>	ABCA1	Cholesterol efflux

Fig. 2 Schematic overview of the most relevant cell types involved in vascular disease, as well as microRNAs that modulate their phenotype in disease progression. *Question mark* indicates indirect target gene

therapy does not alter the progression of atherosclerosis in LDLR-deficient mice [64].

miR-370 and miR-122

The circulating levels of miR-370 and miR-122, but not miR-33a/b, were identified in a recent study to be increased in patients with hyperlipidemia and their expression correlated with coronary artery disease [65]. miR-370 and miR-122 regulates lipid metabolism by controlling cholesterol and fatty acid synthesis and fatty acid β -oxidation [66–70]. Manipulation of miR-370 levels in human hepatic cells (HepG2) resulted in altered fatty acid β -oxidation and triglyceride synthesis, which correlated with changes in the expression levels of *CPT1*, *SREBP1c*, diacylglycerol O-acyltransferase 2 (*DGAT2*), fatty acid synthase (*FAS*) and acetyl-CoA carboxylase (*ACC1*) [70]. Importantly, these changes were abolished by miR-122 ASOs, suggesting that miR-122 expression is induced by miR-370 and that miR-122 is the effector miRNA. In agreement with these results, previous studies have showed that silencing miR-122 resulted in increased hepatic fatty acid β -oxidation and decreased triglyceride and cholesterol synthesis, and a concomitant decrease in circulating

cholesterol [66]. Similar to the mice treated with miR-122 ASOs, circulating cholesterol and triglyceride levels were significantly reduced in mice lacking miR-122 compared with wild-type mice [68, 69]. These results were reproducible in non-human primates, making miR-122 another promising target to manage hypercholesterolemic patients [67]. It is important to stress, however, that the actual physiological targets of miR-122 remain to be established; it is believed that the changes in lipid metabolism mediated by miR-122 are indirect, since none of the above-mentioned genes are direct targets of this miRNA.

Other miRNAs and Lipid Metabolism

Recent studies have identified other miRNAs as integrators of complex lipid regulatory homeostatic pathways. For example, the expression of the human-specific miR-613 is induced by the oxysterol-sensing liver X receptor (LXR), via *SREBP1c*; hsa-miR-613 can subsequently bind to the 3'UTR and repress the expression of LXR, thus providing a negative feedback regulation of LXR-mediated signaling [71]. The intragenic *miR-378/378** is encoded within peroxisome proliferator-activated receptor gamma coactivator 1-beta (*Ppargc1b*) [72], and recent studies show that mice

deficient in this miRNA have increased oxidative metabolic capacity and are protected against diet-induced obesity [73]. Functional targets for miR-378/378* include estrogen-related receptor gamma [74] and insulin-like growth factor 1 receptor [75], which may explain the increased lipogenesis observed following overexpression of miR-378/378* [72]. Other miRNAs including miR-27a/b, miR-143, miR-335, mi-106, miR-125a, and miR-758 have also been implicated in regulating triglyceride and cholesterol metabolism [76–82]. Whether any of these miRNAs will be developed as a drug targets to fight lipid-related disease remains to be established.

Conclusion

Multiple studies have recently shown that miRNAs control critical aspects of lipid metabolism, lipoprotein homeostasis and vascular function (Fig. 2). A growing interest in anti-miR therapies is developing in recent years to treat cardiovascular disease. However, before such new therapeutic approaches are put in place, further studies will be necessary to define the specificity and potency of such anti-miRs, vasculature-specific delivery routes, and the overall changes in gene expression that follow anti-miR treatment.

Acknowledgments Fernández-Hernando Lab and Baldán Lab are supported by grants from the National Institutes of Health (R01HL107953 and R01HL106063 to CF-H and R01HL107794 to AB). We apologize to those whose work could not be cited owing to space limitations.

Disclosure The author reported no potential conflicts of interest relevant to this article.

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