Published in final edited form as:
Expert Rev Mol Diagn. 2011 January ; 11(1): 79–89. doi:10.1586/erm.10.103.

miRNAs: roles and clinical applications in vascular disease

Md Saha Jamaluddin[1], **Sarah M Weakley**[1], **Lidong Zhang**[1], **Panagiotis Kougias**[1], **Peter H Lin**[1], **Qizhi Yao**[1], and **Changyi Chen**[†,1,2]

[1]Michael E DeBakey Department of Surgery, Baylor College of Medicine, One Baylor Plaza, Mail Stop: BCM391, Houston, TX 77030, USA

[2]Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA

Abstract

miRNAs are small, endogenously expressed noncoding RNAs that regulate gene expression, mainly at the post-transcriptional level, via degradation or translational inhibition of their target mRNAs. Functionally, an individual miRNA can regulate the expression of multiple target genes. The study of miRNAs is rapidly growing and recent studies have revealed a significant role of miRNAs in vascular biology and disease. Many miRNAs are highly expressed in the vasculature, and their expression is dysregulated in diseased vessels. Several miRNAs have been found to be critical modulators of vascular pathologies, such as atherosclerosis, lipoprotein metabolism, inflammation, arterial remodeling, angiogenesis, smooth muscle cell regeneration, hypertension, apoptosis, neointimal hyperplasia and signal transduction pathways. Thus, miRNAs may serve as novel biomarkers and/or therapeutic targets for vascular disease. This article summarizes the current studies related to the disease correlations and functional roles of miRNAs in the vascular system and discusses the potential applications of miRNAs in vascular disease.

Keywords

atherosclerosis; biomarker; lipoprotein metabolism; miRNA; therapeutic target; vascular disease; vascular smooth muscle cell

The cardiovascular system is composed of the heart, blood vessels and blood. It is connected intimately with every other organ system, and dysfunction of the cardiovascular system can have devastating downstream effects. The lumen of blood vessels is lined by a monolayer of endothelial cells, which forms the main physical barrier between the blood and vessel wall, controlling the movement of solutes and fluid from the vascular space to the surrounding tissues [1]. Endothelial dysfunction owing to breakdown of the endothelial cell–cell barrier can promote atherogenesis through the increased adherence of leukocytes, monocytes and macrophages, and subendothelial accumulation of cholesterol-bearing lipoproteins [2,3]. Meanwhile, vascular smooth muscle cells (VSMCs) below the endothelium undergo phenotypic modulation from a contractile phenotype to a proliferative state under the influence of mechanical stress, growth factors, inflammatory mediators, such as low-density lipoprotein (LDL) deposition, and leukocyte or monocyte infiltration [4]. Aberrant

[†]Author for correspondence: Tel.: +1 713 798 4401 Fax: +1 713 798 6633 jchen@bcm.tmc.edu .

Financial & competing interests disclosure The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

proliferation of VSMCs and the formation of a neointimal lesion are key pathological processes in a variety of proliferative vascular diseases, such as atherosclerosis, coronary heart diseases, post-angioplasty restenosis and transplantation arteriopathy [5]. During vascular injury, rapid proliferation and growth of vascular cells occur in order to repair the injury, and abnormal repair leads to vascular hyperplasia and neointimal lesion formation; a significant contributor to vascular hyperplasia and neointimal formation is the proliferation of VSMCs [6,7].

Since the first discovery of miRNAs in 1993, the involvement of miRNAs in different aspects of cardiovascular disease has emerged as an important research field. The dysregulation of many individual miRNAs has been linked to the development and progression of cardiovascular disease. Forced expression or suppression of a single miRNA is enough to cause or alleviate pathological change. The role of miRNAs in the pathogenesis of heart and vascular disease points to a possibility of using miRNAs as potential diagnostic biomarkers and/or therapeutic targets for cardiovascular disease. In this article, the miRNAs that have been associated with different aspects of cardiovascular diseases and their molecular mechanisms are discussed, as well as clinical applications of miRNAs as new biomarkers and/or targets for cardiovascular diseases.

Biogenesis & cellular activity of miRNA

miRNAs are highly conserved, single-stranded, noncoding, small RNAs that control cellular function either by degrading mRNAs or by inhibiting their translation. miRNAs can be located in introns of coding or noncoding genes or in exons. miRNAs that have their own promoters are expressed independently, while miRNAs organized in clusters share the same transcriptional regulation [8,9]. miRNAs are produced from longer primary RNA precursors (pri-miRNAs) containing stem–loop structures that are transcribed by RNA polymerase II. These pri-miRNAs are cleaved in the nucleus by the complex of the RNase III enzyme Drosha and its partner DGCR8/Pasha, to form pre-miRNAs of approximately 70 nucleotides in length [10-15]. Pre-miRNAs are transported into the cytoplasm by exportin-5 and subsequently processed by the nuclease Dicer into the 20–24-nucleotide mature miRNAs [16-18].

After processing, the mature miRNA is incorporated into the RNA-induced silencing complex [19], which directs the miRNA to the target mRNA. This leads either to translational repression or degradation of the target mRNA [13]. Whereas mRNA degradation requires a high level of miRNA–target complementarity, translational repression is characterized by low miRNA–target complementarity [20]. It is estimated that approximately a third of all genes are regulated by miRNAs. The complexity of miRNA-dependent gene expression is further extended by the fact that more than one miRNA can cooperatively bind to the same 3′ untranslated region (UTR) [21]. These features of miRNAs facilitate their regulation of important, ubiquitous cellular processes that include apoptosis, senescence and angiogenesis.

Regulation of miRNA biogenesis represents an important site of regulation of miRNA activity, as it involves multiple overlapping interactions between proteins and RNA [22]. For example, similar to protein-coding genes, miRNA genes have promoters that bind transcription factors that regulate their expression. In addition, various co-factors of Dicer, Drosha and their partners augment the miRNA processing machinery; these also regulate the production of miRNAs. Using computational prediction ana lysis, the tumor supressors p53, p73 and p63 have been identified as regulators of the miRNA processing machinery, including Drosha–DGCR3, Dicer–TRBP2 and argonaute proteins [23]. Similarly, signaling by the TGF-β and bone morphogenetic protein (BMP) family lead to the upregulation of

miR-21 and pathologic switching of VSMC into a synthetic phenotype, and this appears to be related to an increase in processing of pre-miR-21 by the Drosha complex [24].

Apoptosis

Numerous miRNAs have been demonstrated to regulate apoptosis experimentally, including the let-7 family, miR-1, miR-1d, miR-7, miR-14, miR-15a, miR-16–1, the miR-17 cluster (miR-17–5p, miR-18, miR-19a, miR-19b, miR-20 and miR-92), miR-21, miR-29, miR-34a, miR-133, miR-146a, miR-146b, miR-148, miR-191, miR-204, miR-210, miR-214, miR-216, miR-278, miR-296, miR-335, miR-Lat and bantam. This list is expected to expand quickly as more studies are performed.

miR-1 promotes oxidative stress-induced apoptosis in cardiomyocytes, mainly by repressing heat-shock protein (HSP)60 and HSP70, and levels of miR-1 are elevated in patients with coronary artery disease [25]. Meanwhile, it has been demonstrated that miR-1 targets both IGF-1 and IGF-1 receptor and, in fact, miR-1 and IGF-1 appear to reciprocally regulate each other [26]. The IGF signaling pathway is an important modulator of cardiac and skeletal muscle growth and survival, and IGF-1 has been demonstrated to be antiapoptotic in a variety of cell types [27].

The apoptosis-regulating miRNAs can be divided broadly into two groups: anti- and pro-apoptotic. This distinction is based primarily on experimental results from particular cell types. For example, inhibition of miR-17–5p and miR-20a induces apoptosis in lung cancer cells, indicating that miR-17–5p and miR-20a may protect these cells from apoptosis and, therefore, are termed antiapoptotic [28]. On the other hand, proapoptotic miRNAs, such as miR-15a and miR-16–1, induce apoptosis in chronic lymphocytic leukemia cells [29]. Each miRNA has the potential to regulate over 1000 protein-coding genes that could well be a mixture of some anti- and pro-apoptotic genes [30]. Whether a miRNA is antiapoptotic or proapoptotic may depend upon the cell-specific expression of genes involved in apoptosis. Increasing experimental evidence suggests that the cell context is very important in determining whether a miRNA is anti- or pro-apoptotic, and a miRNA that is antiapoptotic in a particular cell type may become proapoptotic in another cell type.

Zernecke *et al*. reported the generation of endothelial cell-derived apoptotic bodies during atherosclerosis, which convey paracrine alarm signals to recipient vascular cells, triggering the production of chemokine (C–X–C motif) ligand 12 (CXCL12) [31]. CXCL12 production was mediated by miR-126. Administration of apoptotic bodies or miR-126 limited atherosclerosis, promoted the incorporation of stem cell antigen-1 (Sca-1)-positive progenitor cells, and conferred features of plaque stability in different mouse models of atherosclerosis, indicating that miR-126 has atheroprotective effects [31].

Senescence

Cellular senescence is a mechanism to inhibit the growth of mammalian cells after oncogenic activation or in response to damage or stress [32]. Increased numbers of senescent endothelial cells are found in mature atherosclerotic plaques, vessels from diabetic patients, postangioplastic restenotic vessels, coronary vessels of patients with ischemic heart disease and in hypertensive patients [33]. One of the candidate miRNAs involved in vascular senescence is miR-34a [34]. miR-34a is highly expressed in primary endothelial cells and its expression increases in senescent human umbilical vein endothelial cells (HUVECs) and the hearts and spleens of older mice. Overexpression of miR-34a was found to induce endothelial cell senescence and also suppress cell proliferation by inhibiting cell cycle progression. Silent information regulator 1 (Sirt1) has been found to be a target of miR-34a. Initially identified as a longevity gene, *Sirt1* has recently been implicated as a

novel modulator of vascular endothelial cell homeostasis, playing a key role in angiogenesis through the deacetylation of forkhead box O transcription factor 1 (FoxO1) [35,36]. Overexpression of miR-34a inhibits Sirt1 protein expression, while miR-34a knockdown enhances Sirt1 expression. miR-34a triggers endothelial senescence, in part, through Sirt1, since forced expression of Sirt1 blocks the ability of miR-34a to induce senescence. miR-217 also regulates the expression of Sirt1 [37]. Aging is a major risk factor for the development of atherosclerosis and coronary artery disease, and miR-217 is progressively expressed in endothelial cells with aging [38].

Angiogenesis

Angiogenesis is an important cellular process, both in normal physiology and in pathologic states, such as ischemic heart disease and cancer. miRNAs appear to play a critical role in angio genesis. This has been demonstrated by genetic silencing of Dicer, the rate-limiting enzyme required for the normal processing of mature miRNA. Silencing of Dicer *in vivo* reduces angiogenesis, even with stimulation by exogenous VEGF, tumors, limb ischemia and wound healing [39]. Silencing of Dicer has also been demonstrated to lead to a reduction in markers of angiogenesis *in vitro* and *in vivo*; this appeared to be through regulation of the angiogenesis inhibitor thrombospondin-1 (TSP-1) via miRNA let-7 family members [40].

The majority of miRNAs have been found to be downregulated in endothelial cells [40]. In HUVECs, meanwhile, 15 miRNAs have been found to be highly expressed and are thought to regulate several angiogenic factors, including Flt-1, Nrp-2, Fgf-R and c-Met, based on prediction algorithms [38]. HUVECs are useful for studying angiogenesis because of their formation of tubes, which are structures that resemble capillaries. It has been demonstrated in HUVECs that miR-221 and miR-222 modulate angiogenesis through their interaction with the 3′UTR of c-kit, which is a receptor for stem cell factor (SCF), itself being a proangiogenic factor [38]. miR-221 has been implicated in inhibiting endothelial cell tube formation and migration; this appears to be mediated by increased expression of the antiangiogenic homeobox gene *GAX*, through the regulation of *ZEB2* by miR-221 [41].

The pathologic angiogenesis involved in tumor formation has also been investigated. Recently, miR-132 has been proposed to be an ‘angiogenic switch’ in tumor formation; this miRNA is highly upregulated in conditions of angiogenesis but is absent in normal endothelium, and miR-132 appears to suppress p120RasGAP, leading to increased Ras activity and neovascularization. This pattern was also observed in human tumors in comparison with normal tissue. Meanwhile, administration of anti-miR-132 reversed these effects [42]. miR-126 has also been found to modulate angiogenesis in a positive way; its deletion leads to vascular leakage, hemorrhage and early death in mice; these effects appear to be mediated through repression of Spred-1, which is a negative regulator of MAPK signaling [43]. miR-126 appears to increase the response of endothelial cells to VEGF, promoting angiogenesis *in vivo* and *in vitro* in zebrafish [44]. Meanwhile, administration of VEGF to human vascular endothelial cells has been demonstrated to increase the level of several miRNAs in human tumors, including miR-155, miR-191, miR-21, miR-18a, miR-17–5p and miR-20a; these have been associated with tumor growth, survival and angiogenesis [39].

Several authors have reviewed the literature on all angiogenesis-related miRNAs, including miR-27b, miR-126, miR-17–92, let-7, miR-17–92 cluster, miR-130a, miR-210, miR-378 and miR-296, which are thought to have potential angiogenic effects; meanwhile miR-221/222, miR-328, miR-92a and miR-214 have been found to be antiangiogenic [45,46]. The effects of these miRNAs on endothelial biology and angiogenesis have been

identified in a variety of settings, both in cultured endothelial cells *in vitro* and in ischemia-induced angiogenesis *in vivo*, using limb ischemia and myocardial infarction animal models [47,48].

Atherosclerosis

Atherosclerosis is a pathological process in which deposits of fatty compounds, cholesterol, cellular waste products, calcium and other substances accumulate in the inner lining of an artery, forming plaques. It usually affects large- and medium-sized arteries. Plaques can grow large enough to significantly reduce the blood flow through an artery. The most serious damage may occur when plaques become fragile and rupture. Plaque rupture can trigger vascular thrombosis, which can block blood flow, or plaques can embolize, leading to myocardial infarction, stroke or acute or chronic limb ischemia. The major risk factors for atherosclerosis include elevated levels of cholesterol and triglyceride in the blood, high blood pressure, tobacco smoking, diabetes, obesity and physical inactivity. The first major event in the progression of the early atheroma is the loss of endothelial integrity. Endothelial dysfunction then facilitates the subendothelial accumulation of cholesterol-bearing lipoproteins and compromises vasodilation.

In a recent study, individuals with atherosclerosis, as defined by coronary artery disease (CAD), showed a significantly higher expression of miR-221 and miR-222 in endothelial progenitor cells (EPCs) compared with non-CAD individuals [49]. EPCs play an important role in the maintenance of vascular integrity. Atorvastatin treatment in CAD groups increased EPC numbers and decreased miR-221/222 levels, indicating that miR-221/222 levels were inversely related to EPC levels, as CAD individuals had significantly lower EPC numbers [49].

Inflammatory environment in atherosclerosis

A key step in the development of chronic inflammatory atherosclerotic disease is the migration of circulating monocytes into the subendothelial space and their differentiation into macrophages. A recent study showed that miR-125a–5p mediates lipid uptake and decreases the secretion of some inflammatory cytokines, including IL-2, IL-6, TNF-α and TGF-β in oxidized LDL-stimulated monocyte-derived macrophages [50]. The target gene of miR-125a-5p has been found to be *ORP9*, which has diverse roles in the regulation of lipid metabolism, including vesicle transport, as well as cell cycle regulation and differentiation [51,52]. Meanwhile, miR-10a has been implicated in contributing to the proinflammatory endothelial phenotype observed in atherosclerosis formation. In swine, atherosusceptible regions of the arterial tree had lower levels of endothelial miR-10a than elsewhere. Homeobox A1 (HOXA1) is a known downstream target of miR-10a, and it was found to be upregulated in the same areas. Meanwhile, miR-10a knockdown in endothelial cells led to an increase in inflammatory biomarkers. Furthermore, miR-10a was found to interact with the 3′UTRs of *MAP3K7* and β*TRC*, and these were upregulated when miR-10a was knocked down, which forms further evidence that miR-10a has a regulatory role with respect to atherogenic inflammation [53].

Vascular smooth muscle cells

The plasticity of VSMCs plays an important role during development and in vascular pathologies, such as atherosclerosis and restenosis. VSMCs form layers within the vessel wall and control blood flow by contracting or relaxing in response to external stimuli. Under normal physiologic conditions, VSMCs rarely proliferate. However, in the presence of injury, LDL deposition or in response to inflammatory stimuli on the vascular endothelium, VSMCs begin to grow and divide. Over time, endothelial function deteriorates, and aberrant

proliferation of VSMCs can lead to pathologic changes in the vascular walls [54]. Dedifferentiation of VSMCs to a proliferative state is believed to be critical for the response to vascular injury, and this process has also been correlated with multiple vascular proliferative diseases, including restenosis after balloon angioplasty or stenting, atherosclerosis and transplant vasculopathy [4,55,56]. Recent studies have indicated that several miRNAs are highly expressed in the vascular system and are involved in the control of proliferation and differentiation of VSMCs. For example, signaling by TGF-β and BMP family members is thought to be a critical modulator of the switching of VSMC from contractile to synthetic state via upregulation of several miRNAs, and miR-21 has been found to mediate the effects of BMP signaling on VSMC differentiation [24].

Vascular smooth muscle cells are rich in miR-145 and miR-143, both of which play significant roles in regulating the phenotypic switching from a contractile to proliferative states. Overexpression of miR-145 or miR-143 promotes the differentiation and inhibits proliferation of cultured VSMCs [7,57]. miR-143- and miR-145-deficient VSMCs, on the other hand, fail to demonstrate a contractile phenotype in response to vasopressive stimuli [54,58]. miR-143/145-mutant mice show significantly decreased numbers of contractile VSMCs and remarkably increased numbers of proliferative VSMCs in the aorta and the femoral artery [7,54,57-59]. VSMCs within miR-143/145-mutant arteries demonstrate prosynthetic morphological features and significant downregulation in the expression of VSMC-specific differentiated markers [54,58].

miR-143/145 also inhibit the formation of podosomes, which are actin-rich membrane protrusions involved in the migration of VSMCs. Meanwhile, PDGF, a potent stimulator of VSMC migration, can downregulate the expression of miR-143/145, inducing podosome formation [60]. This appears to be mediated through the activity of Src and p53. Targets of miR-143 and miR-145 have been identified; miR-143 reduced intracellular levels of PDGF receptor α (PDGF-Rα) and the serine/threonine kinase protein kinase Cε (PKCε), which is involved in cell migration and proliferation, while miR-145 decreased the level of actin-bundling protein fascin without affecting mRNA levels. Dysregulation of the miR-143 and miR-145 regulatory genes is probably involved in the aberrant VSMC migration and proliferation encountered during vascular disease formation [60].

The expression of miR-221 and miR-222 is upregulated and localized in VSMCs in the injured vascular walls in a rat model of angioplasty, and miR-221 and miR-222 appear to be important regulators of VSMC proliferation and neointimal hyperplasia. In cultured VSMCs, the expression of miR-221 and miR-222 is increased in response to PDGF or serum. Knockdown of miR-221 and miR-222 by 2′OMe-miR-222, the inhibitor for both miR-221 and miR-222, results in decreased VSMC proliferation *in vitro*. Meanwhile, p27 (*Kip1*) and p57 (*Kip2*) were identified as two target genes that were involved in miR-221- and miR-222-mediated effects on VSMC growth using gain-of-function and loss-of-function studies. Finally, knockdown of miR-221 and miR-222 was found to suppress VSMC proliferation and neointimal lesion formation after angioplasty in rat carotid arteries [61].

Lipoprotein metabolism

Lipids, such as triglycerides and cholesterol, are insoluble in circulating plasma. In order to carry these lipids through plasma, proteins called apolipoproteins bind with lipids to form lipoproteins, such as LDL and high-density lipoprotein (HDL). The primary functions of lipoproteins are the delivery of neutral lipids, such as triglycerides and, to a lesser degree, cholesterol, to peripheral cells; they also remove excess cellular cholesterol by the reverse cholesterol transport pathway [62]. As such, lipoproteins are critical mediators of atherosclerosis formation. miR-33 appears to regulate both HDL biogenesis in the liver and

cellular cholesterol efflux [63-65]. miR-33 is an intronic miRNA located within the gene encoding sterol-regulatory element-binding factor-2, a transcriptional regulator of cholesterol synthesis, and miR-33 modulates the expression of genes involved in cellular cholesterol transport. miR-33 appears to be regulated by dietary cholesterol *in vivo* and have several roles in cholesterol homeostasis [64]. It appears to regulate both HDL biogenesis in the liver and cellular cholesterol efflux. miR-33 targets the 3′UTR of the adenosine triphosphate-binding cassette (ABC) transporter ABCA1 in mouse peritoneal macrophages and in human cells [64,65]; as a result, atherogenic cholesterol efflux to apolipoprotein A1 is reduced [64]. Similarly, in a mouse model, lentiviral delivery of miR-33 represses ABCA1 expression in the liver, leading to a reduction in circulating HDL levels, while mice expressing anti-miR-33 *in vivo* demonstrate increased plasma HDL levels [64,65]; however, there have been conflicting results regarding the effect of miR-33 silencing on hepatic expression of ABCA1, with one investigation showing an increase in hepatic ABCA1 [64] and another showing no significant change [65]. Clearly, miR-33 is a promising target for treating the abnormalities in lipoprotein metabolism that frequently contribute to atherosclerosis.

miR-122 also has a direct role in cholesterol metabolism [66-68]. The expression of miR-122 is highly restricted to the liver. Overexpression of miR-122 with adenovirus increases the expression of cholesterol-synthesis genes (Hmgrcs1, Sqle and Dhcr7) in the liver, which in turn increases cholesterol synthesis [68]. Silencing of miR-122 expression in hepatocytes downregulates genes implicated in cholesterol biosynthesis and triglyceride metabolism, increasing hepatic fatty acid oxidation and reducing plasma cholesterol, hepatic fatty acid and cholesterol synthesis. However, the direct targets of miR-122 that confer these effects on the cholesterol biosynthetic and metabolic pathways are still unknown [69]. Meanwhile, miR-125a-5p has been found to mediate lipid uptake in oxidized LDL-stimulated monocyte-derived macrophages [50].

Human endothelial nitric oxide synthase regulation

Human endothelial nitric oxide (NO) synthase (eNOS) is an enzyme that is constitutively expressed, mainly in endothelial cells. eNOS, in the form of homodimers, catalyzes L-arginine oxidation to gene rate L-citrulline and NO in the presence of several cofactors [70]. NO, meanwhile, is an antiatherogenic molecule. While a large amount of data exist regarding the regulation of eNOS enzyme activity, relatively little information is available about the role of miRNAs in regulating eNOS expression.

Shear stress is an important activator of eNOS expression [71] and has been demonstrated to increase the expression of 13 miRNAs in HUVECs. Among them, the upregulation of miR-21 was highest. Western blot ana lysis demonstrated that PTEN, a known target of miR-21, was downregulated in HUVECs exposed to shear stress or transfected with pre-miR-21. Importantly, HUVECs overexpressing miR-21 demonstrated decreased apoptosis and increased eNOS phosphorylation and NO production [72]. These data demonstrate that shear stress regulates the expression of miR-21 in endothelial cells, which, in turn, influences endothelial biology by decreasing apoptosis and activating the NO pathway.

Ginsenoside-Rg1 is one of the active components of ginseng, and it is an angiogenesis inducer of eNOS expression. This effect appears to be mediated by a ginsenoside-Rg1-induced downregulation of miR-214, leading to increased eNOS expression. Meanwhile, computational prediction has revealed that miR-214 may potentially target eNOS, as sequence alignment showed that miR-214 is partially complementary to the RNA sequence extending from nucleotide 168 to 189 within the 3′-UTR of eNOS mRNA. In addition, transfection of a miR-214 precursor mimic (pre-miR-214) repressed expression of eNOS in

HUVECs, while transfection of an antagomiRNA antisense to mature miR-214 (AS-miR-214) enhanced eNOS expression [73]. Overall, these data strongly suggest that miR-214 is involved in Rg1-induced eNOS expression and angiogenesis in HUVECs.

Arterial remodeling

miR-222 has been demonstrated to be involved in inflammation-mediated vascular remodeling [74]. miR-222 targets STAT5A and negatively correlates with STAT5A expression in human endothelial cells from advanced neovascularized atherosclerotic lesions. Furthermore, upregulation of STAT5A due to IL-3/basic FGF (bFGF)-induced downregulation of miR-222 controls endothelial cell proliferation and migration and, therefore, facilitates intraplaque neovascularization during atherosclerosis. In fact, in advanced lesions, there was an increased proliferation rate of endothelial cells lining vessels, which correlated with a diminished expression of miR-222. Thus, miR-222 acts as a negative regulator of neovascularization through post-transcriptional regulation of STAT5A.

miRNA as a biomarker for coronary artery disease

Levels of circulating miRNAs are being studied extensively as a novel class of biomarker for various diseases, especially cancer [75]. miRNA in clinical plasma samples is stable with respect to freezing and rethawing [76] and appears to be protected from endogenous RNase activity [77]. miRNA has been found to circulate in the plasma in microvesicles, platelets and peripheral blood mononuclear cells (PBMCs) [78], and in healthy human subjects, it has been determined that over 250 miRNAs can be detected in the circulation [79]. However, the lack of standardized control values for circulating miRNA levels is presently a limitation in the interpretation of data in which miRNA is investigated as a biomarker [75]. In addition, in this emerging field, small sample sizes often limit the immediate translational applicability of the data. Nonetheless, these issues should be addressed shortly, and at present, exciting data exist to suggest that miRNAs may prove to be valuable biomarkers in the detection of cardiovascular disease.

Expression of miR-146a is upregulated in the PBMCs of patients with acute coronary syndrome (ACS), including stable angina and acute myocardial infarction (AMI) [80]. Overexpression of miR-146a positively correlates with the plasma concentrations of proinflammatory cytokines, such as IFN-γ, TNF-α and NF-κB p65, a critical transcription factor in atherosclerosis. Overexpression of miR-146a in PBMCs significantly upregulates the function of Th1 cells and is related to the onset and progression of ACS. By contrast, miR-146a inhibitor reduced this effect [80]. Thus, miR-146a may represent a novel regulatory factor in ACS patients and could be a new therapeutic target for atherosclerosis and ACS.

Several other recent studies have examined levels of circulating miRNAs in patients with AMI [79,81-83]. Expression of miR-1, which was found previously to be upregulated in ischemic cardiac tissue, was found to be significantly higher in plasma from AMI patients compared with non-AMI subjects; the level of miR-1 dropped to normal upon discharge [79,81,82]. The levels of miR-208a, miR-133a and miR-499 were also elevated in AMI patients in comparison with non-AMI patients, and there was no detectable miR-208 in healthy subjects [83,84]. However, of the four miRNAs, only miR-208a appears to be expressed in a cardiac-specific manner [83,85]; plasma elevations of miR-1, miR-133a and miR-499 may occur after skeletal muscle injury, which may limit their use as biomarkers for AMI [83]. Meanwhile, miR-499 has been described in other investigations to be produced almost exclusively in the heart [84]. In addition, it has been demonstrated that miR-1 and miR-133a, while highly expressed in skeletal muscle, do not increase in a mouse model of skeletal muscle damage due to acute hind-limb ischemia [79]. In light of these findings, it

has been hypothesized that under conditions of cardiac injury, specific miRNAs in the systemic circulation may reflect myocardial damage and prove to be valuable bio markers for AMI. However, again, these studies will first need to be extended to larger populations and the cardiac specificity of the individual miRNAs delineated clearly in order to achieve clinical significance.

Levels of circulating miRNAs have also been examined in patients with stable CAD in comparison with healthy subjects. Most vascular-derived miRNAs, including miR-126 and the miR-17–92 cluster, were actually downregulated in patients with CAD; this may be due to uptake into atherosclerotic lesions within the vasculature of these patients. Meanwhile, in the same patients, there was a trend towards increased levels of skeletal/cardiac muscle-derived miRNAs, such as miR-133a and miR-208a. While these patients were not losing active myocardial tissue, as evidenced by baseline troponins, the elevation in muscle-derived miRNAs may be due to a ‘low grade of cardiac myocyte injury’ [86].

Stroke

Stroke is the second most common cause of death and consumes approximately 2–4% of total healthcare costs worldwide. The majority of strokes are ischemic and may result from thrombosis, emobolism or global hyperperfusion. A search for biomarkers to aid in the diagnosis of stroke has commenced as well. Through array ana lysis of 836 miRNAs in blood samples in stroke patients, eight miRNAs (let-7f, miR-126, miR-1259, miR-142–3p, miR-15b, miR-186, miR-519e and miR-768–5p) were poorly expressed across the three subtypes of stroke (large artery, small artery and cardioembolic stroke), while 17 miRNAs (let-7e, miR-1184, miR-1246, miR-1261, miR-1275, miR-1285, miR-1290, miR-181a, miR-25*, miR-513a-5p, miR-550, miR-602, miR-665, miR-891a, miR-933, miR-939 and miR-923) were highly expressed in these subtypes [87]. However, it is not clear whether these miRNAs will be useful as biomarkers for stroke.

Pulmonary hypertension

Severe pulmonary arterial hypertension (PAH) is characterized by significant increases in pulmonary artery pressures to levels present in the systemic circulation. PAH is a major determinant of morbidity and mortality in cardiopulmonary disease. The pathogenesis of PAH revolves around excessive vasoconstriction and/or abnormal pulmonary vascular remodeling. Recent experimental evidence has linked the pulmonary vascular disease present in PAH to an abnormal proliferative vascular cell pheno type, which is also characterized by resistance to endothelial and/or VSMC apoptosis [88]. Caruso *et al*. screened and profiled miRNA signatures in the rat lung, and compared them longitudinally with rats exposed to either chronic hypoxia or monocrotaline injuries at 2, 7 and 21 days. Monocrotaline, a pyrrolizidine alkaloid and a toxic plant constituent, causes PAH, right ventricular hypertrophy and pathological changes in the pulmonary vasculature. The study showed that the expression of Dicer during the onset of PAH after hypoxia was reduced. miR-22, miR-30 and let-7f were downregulated, whereas miR-322 and miR-451 were upregulated significantly during the development of PAH in both hypoxic and monocrotaline models. miR-21 and let-7a were significantly reduced only in monocrotaline-treated rats [89]. Chen *et al*. investigated the involvement of miR-759 in chronic thromboembolic pulmonary hypertension (CTEPH). One of the leading causes of severe pulmonary hypertension, CTEPH is characterized by persistent pulmonary embolism that increases pulmonary vascular resistance, resulting in pulmonary hypertension and subsequent right ventricular heart failure. The 3′UTR of *FGA* was found to interact with miR-759, and a 28-bp deletion polymorphism at this site was found to be more frequent in

patients with CTEPH [90]. Fibrinogen, also called factor I, plays an important role in blood clotting, fibrinolysis and inflammation [91].

Neointimal hyperplasia & vascular response to injury

Neointimal lesion formation is a common pathological event seen in diverse proliferative vascular diseases, such as atherosclerosis, coronary heart disease, post-angioplasty restenosis and transplantation arteriopathy. Neointimal hyperplasia has also generally been accepted as the main feature of vascular repair responses to various injuries and several investigations have suggested that miRNAs are important mediators of this process.

Post-angioplasty stenosis

Multiple miRNAs are differentially expressed in rat carotid arteries demonstrating neointimal growth after angioplasty [92]. Among the aberrantly expressed miRNAs, miR-21 expression underwent a more than fivefold increase in balloon-injured arteries compared with normal control vessels. Meanwhile, knockdown of miR-21 in balloon-injured rat carotid arteries significantly inhibited the growth of neointimal lesions. Antiapoptotic and proliferative effects on VSMCs are thought to be the major mechanisms whereby miR-21 mediates vascular neointimal growth in cultured VSMCs *in vitro* and in rat carotid arteries *in vivo* [93]. miR-21 targets mainly *PTEN* and *PDCD4* when exerting its effect on VSMCs. In addition, reactive oxygen species (ROS) contribute to the pathogenesis of atherosclerosis and restenosis after angioplasty [94]. miR-21 is highly expressed in VSMCs treated with ROS, such as hydrogen peroxide [95]. In the rat carotid artery balloon-injury model, neointimal hyperplasia of injured artery was significantly repressed when antisense oligonucleotide-mediated miRNA depletion led to downregulation of miR-21 [93].

Following vascular injury, miR-221 and miR-222 increase in the balloon-injured carotid arteries and localize primarily in VSMCs of the vascular wall [61]. Knockdown of miR-221 and miR-222 can inhibit VSMC proliferation and neointimal thickening in rat carotid artery after vascular injury [61]. In another vascular-injury model, miR-143 and miR-145 expression was significantly decreased in ligated carotid arteries compared with control arteries [57]. Furthermore, miR-145 was decreased to nearly undetectable levels in mouse arteriosclerotic lesions [57]. Meanwhile, miR-143/145-mutant mice showed a significant increase in neointimal lesion formation with large amounts of VSMCs, macrophages and deposits of amorphous collagen I in the femoral arteries [54]. Several investigations have shown that introduction of miR-145 or miR-143/145 into injured rat carotid arteries via adenovirus-mediated gene transfer leads to a significant reduction in neointimal lesion formation [7,58]; when miR-145 was administered, the reduced lesion formation was accompanied by upregulation of VSMC differentiation markers [7]. The effect of miR-143 and miR-145 on neointimal hyperplasia is also reported in a mouse model of vascular injury [59]. Taken together, these data indicate that miR-143 and miR-145 play pivotal roles in the control of the contractile phenotype of VSMCs and the response of the vascular wall to injury, which has important therapeutic implications.

Proliferative signal transduction pathways

Different types of signal transduction pathways are involved in miRNA-mediated effects on the vascular system. One example is the previously mentioned involvement of TGF-β signaling pathways during the transition of VSMCs from a contractile to a synthetic phenotype [24,96]. Modulation of the VSMC phenotype from a quiescent contractile phenotype to a proliferative synthetic phenotype has been implicated in vascular injury repair, as well as in the pathogenesis of vascular proliferative diseases. BMP and TGF-β signaling pathways promote a contractile phenotype, while the PDGF–BB signaling

pathway promotes a switch to the synthetic phenotype. PDGF–BB can induce miR-24, which, in turn, leads to downregulation of Tribbles-like protein-3 (Trb3) [97]. Repression of Trb3 coincides with the reduced expression of Smad proteins and a decrease in BMP and TGF-β signaling, promoting a synthetic phenotype in VSMCs. Inhibition of miR-24 by antisense oligonuclotides abrogates the downregulation of Trb3 as well as the prosynthetic activity of the PDGF signaling pathway. Both miR-21 and miR-24 have been implicated in the pathways whereby PDGF and TGF-β control the VSMC phenotype [24,97].

miRNAs as therapeutic targets

Ongoing research shows that miRNAs are involved in almost all types of cellular processes, making them ideal targets for therapeutic use. Multiple miRNAs are involved in the development of both human and animal vascular diseases as they regulate vascular cell differentiation, migration, proliferation and apoptosis through their target genes. Several *in vivo* animal studies have revealed promising results in treating vascular disease. Thus, miRNAs may represent both new biomarkers and new therapeutic targets for diverse vascular diseases.

Vascular diseases are complex, multifactorial diseases, in which many genes are involved. Meanwhile, one miRNA can have multiple targets, and one gene can be regulated by several miRNAs. Keeping this in mind, miRNA-based therapy may have both advantages and disadvantages. miRNAs that have only a single target gene should be easy to suppress using anti-miRNA technology, which represents an advantage. However, suppression of miRNAs that have multiple target genes will affect several genes and might induce some unexpected side effects; this, then, could be a disadvantage [98]. Since miRNAs are endogenous, restoration of aberrantly expressed miRNAs, both upregulated and downregulated, to physiological levels should not have major unexpected side effects. Therefore, strategies for miRNA-based therapies are based on either the restoration of suppressed genes by reducing or inhibiting specific miRNAs or the delivery/overexpression of specific miRNAs to suppress the target gene responsible for disease formation.

As we are still in the early stages of developing miRNA-based therapy, further studies need to be performed carefully before it can be used in the clinic. First, the critical miRNAs responsible for the development of vascular diseases should be identified definitively. Second, the detailed cellular and molecular mechanisms of these critical miRNAs in the prevention and treatment of vascular diseases should be elucidated. Third, in addition to the biological effects of these miRNAs on endothelial cells and VSMCs, their effects on other vascular disease-related cellular processes should be identified. Fourth, although methods are available to downregulate miRNA *in vivo*, technology for upregulating miRNA in the vascular walls *in vivo* requires further development. Finally, the potential side effects of miRNA-based therapy should be studied before application in patients.

There are several strategies by which miRNAs can be deactivated or destroyed effectively. The fact that miRNAs bind to their target mRNAs by Watson–Crick base pairing indicates that the usage of an oligonucleotide that is complementary to the miRNA and effectively competes with the mRNA target (i.e., anti-miRNAs) represents a potentially effective way of inactivating pathological miRNAs; thus, downregulation of important target genes that promote gene expression could be avoided [66-68]. Alternatively, miRNA mimics (synthetic, non-natural nucleic acids that can bind to the unique sequence of target mRNAs in a gene-specific manner) may induce target downregulation and thereby diminish gene expression; however, this approach has not been tested *in vivo* [99,100]. The use of anti-miRNAs in cultured cells has been successful. Chemical modification of miRNA inhibitors is key in the development for use *in vivo*.

Three different types of chemical modifications have been carried out to enact the inhibition of miRNA function *in vivo*. The first class of anti-miRNAs is conjugated to cholesterol (antago-miRNA) to facilitate cellular uptake. Other classes use oligonucleotides with locked nucleotides acid (anti-miRNAs) or 2′-*O*-methoxyethyl phosphorothioate modifications. Antagonism of miR-122 in mouse liver using these three classes of antimiRNAs in three independent studies revealed that miR-122 antagonism led to reduced plasma cholesterol levels in cardiovascular diseases, including hypertrophy, heart failure, cardiac injury, arrhythmia and atherosclerosis [66-68]. Therefore, these approaches to manipulate the expression of miRNAs, either positively or negatively, can potentially be used for therapeutic purposes. For those miRNAs that are downregulated in disease states, a forced miRNA re-expression strategy may be used to recover miRNA expression; conversely, an anti-miRNA strategy may be employed to suppress the expression of upregulated miRNAs. Although the targeting of miRNAs represents a promising therapeutic strategy, substantial preclinical work still needs to be conducted in order to identify precise targets among the many possible targets in vascular disease.

To date, more than 800 human miRNAs have been identified (miBase) and work continues in the search for new miRNAs. miRNAs as important regulators of gene expression represent a relatively recent discovery. It appears that miRNAs are key regulators in almost all cellular processes and in many human diseases. In this article, we have summarized the role of miRNAs in the regulation of vascular disease and the possibilities of using miRNA-directed therapies as a treatment option for vascular diseases associated with the aberrant expression of miRNAs. The biggest obstacle to the development of miRNA-based clinical therapy is how to efficiently deliver miRNA mimics and inhibitors to target organs or cells. With rigorous fundamental and clinical studies, a clearer understanding about miRNAs as biomarkers and targets for cardiovascular disease will develop.

Expert commentary

With the recent surge of research into miRNAs, our understanding of the role of miRNAs in vascular biology is rapidly evolving and several strategies have been developed to suppress or overexpress specific miRNAs. The functions of aberrantly expressed miRNAs in the vascular system and their mechanisms of actions, particularly their target genes and signaling pathways, need to be studied in detail in order to efficiently develop biomarker applications and therapeutic approaches. The combination of computational ana lysis, bioinformatics and detailed molecular mechanism ana lysis using *in vitro* cell culture and *in vivo* knockout ana lysis in animals will be needed for the identification of miRNA target genes. Of note, again, one miRNA may have several target genes or one gene might be regulated by several miRNAs. Therefore, targeting a specific miRNA may have unexpected effects in the experimental system. Several strategies, including anti-miRNAs, miRNA mimics, miRNA inhibitors and miRNA sponges, have been developed to suppress pathological miRNAs. However, stringent research is needed to efficiently deliver these miRNA inhibitors to the specific target organs or cells. Finally, substantial preclinical work still needs to be conducted to identify precise target genes among the many predicted target genes in vascular diseases.

Five-year view

Results from recent studies demonstrate a broad role of miRNAs in cardiovascular development and disease formation. Determining miRNA regulatory circuits and defining the mechanisms whereby miRNAs control the expression of target genes will be key to future miRNA-based therapeutic applications towards cardio vascular disease prevention. The specific recognition of a miRNA to its target 3′UTR is highly regulated; yet, the

mechanisms that regulate this recognition are still under investigation. One important research direction is the impact of genomic variation in miRNA genes on miRNA function and the contribution of this individual variability to disease formation. Currently, more than 800 miRNAs have been discovered and many new miRNAs are being discovered. Therefore, it is certain that many new and unanticipated roles of miRNAs in the control of cardiovascular functions will be discovered and will assist clinicians and researchers in developing potential therapeutic applications.

Acknowledgments

This work was partially supported by a NIH grant (R01HL083471 to Changyi Chen) and by the Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA. Sarah M Weakley and Lidong Zhang were supported by a NIH training grant (T32HL083774).

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Stevens T, Garcia JG, Shasby DM, Bhattacharya J, Malik AB. Mechanisms regulating endothelial cell barrier function. Am. J. Physiol. Lung Cell. Mol. Physiol. 2000; 279(3):L419–L422. [PubMed: 10956614]
2. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. J. Am. Coll. Cardiol. 2003; 42(7):1149–1160. [PubMed: 14522472]
3. Endemann DH, Schiffrin EL. Endothelial dysfunction. J. Am. Soc. Nephrol. 2004; 15(8):1983–1992. [PubMed: 15284284]
4. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol. Rev. 2004; 84(3):767–801. [PubMed: 15269336]
5. Zhang C. MicroRNAs: role in cardiovascular biology and disease. Clin. Sci. (Lond.). 2008; 114(12): 699–706. [PubMed: 18474027]
6. Zhang C. MicroRNA-145 in vascular smooth muscle cell biology: a new therapeutic target for vascular disease. Cell Cycle. 2009; 8(21):3469–3473. [PubMed: 19829088]
7. Cheng Y, Liu X, Yang J, et al. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. Circ. Res. 2009; 105(2):158–166. [PubMed: 19542014] •• Describes the expression and function of miR-145 in vascular smooth muscle cells *in vitro* and *in vivo*.
8. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004; 23(20):4051–4060. [PubMed: 15372072]
9. Altuvia Y, Landgraf P, Lithwick G, et al. Clustering and conservation patterns of human microRNAs. Nucleic Acids Res. 2005; 33(8):2697–2706. [PubMed: 15891114]
10. Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003; 425(6956):415–419. [PubMed: 14508493]
11. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the microprocessor complex. Nature. 2004; 432(7014):231–235. [PubMed: 15531879]
12. Gregory RI, Yan KP, Amuthan G, et al. The Microprocessor complex mediates the genesis of microRNAs. Nature. 2004; 432(7014):235–240. [PubMed: 15531877] • Describes human Drosha, a component of two multiprotein complexes that mediate the genesis of miRNA from the primary miRNA transcript.
13. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116(2):281–297. [PubMed: 14744438] •• Summarizes basic information about miRNAs.

14. Han J, Lee Y, Yeom KH, et al. The Drosha–DGCR8 complex in primary microRNA processing. Genes Dev. 2004; 18(24):3016–3027. [PubMed: 15574589]
15. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev. 2003; 17(24):3011–3016. [PubMed: 14681208]
16. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA. 2004; 10(2):185–191. [PubMed: 14730017]
17. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004; 303(5654):95–98. [PubMed: 14631048]
18. Hutvagner G, McLachlan J, Pasquinelli AE, et al. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science. 2001; 293(5531):834–838. [PubMed: 11452083]
19. Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ. Argonaute2, a link between genetic and biochemical analyses of RNAi. Science. 2001; 293(5532):1146–1150. [PubMed: 11498593]
20. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003; 115(7):787–798. [PubMed: 14697198] • Describes the prediction of regulatory target genes for conserved vertebrate miRNAs.
21. Doench JG, Petersen CP, Sharp PA. siRNAs can function as miRNAs. Genes Dev. 2003; 17(4): 438–442. [PubMed: 12600936]
22. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat. Rev. Genet. 2010; 11(9):597–610. [PubMed: 20661255]
23. Boominathan L. The tumor suppressors p53, p63, and p73 are regulators of microRNA processing complex. PLoS ONE. 2010; 5(5):e10615. [PubMed: 20485546]
24. Davis BN, Hilyard AC, Lagna G, Hata A. SMAD proteins control Drosha-mediated microRNA maturation. Nature. 2008; 454(7200):56–61. [PubMed: 18548003]
25. Xu C, Lu Y, Pan Z, et al. The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. J. Cell Sci. 2007; 120(Pt 17):3045–3052. [PubMed: 17715156]
26. Elia L, Contu R, Quintavalle M, et al. Reciprocal regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions. Circulation. 2009; 120(23):2377–2385. [PubMed: 19933931]
27. Parrizas M, Saltiel AR, LeRoith D. Insulin-like growth factor 1 inhibits apoptosis using the phosphatidylinositol 3′-kinase and mitogen-activated protein kinase pathways. J. Biol. Chem. 1997; 272(1):154–161. [PubMed: 8995241]
28. Matsubara H, Takeuchi T, Nishikawa E, et al. Apoptosis induction by antisense oligonucleotides against miR-17–5p and miR-20a in lung cancers overexpressing miR-17–92. Oncogene. 2007; 26(41):6099–6105. [PubMed: 17384677]
29. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc. Natl Acad. Sci. USA. 2005; 102(39):13944–13949. [PubMed: 16166262]
30. Miranda KC, Huynh T, Tay Y, et al. A pattern-based method for the identification of microRNA binding sites and their corresponding heteroduplexes. Cell. 2006; 126(6):1203–1217. [PubMed: 16990141] • Describes a method for identifying miRNA binding sites and their corresponding heteroduplexes.
31. Zernecke A, Bidzhekov K, Noels H, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci. Signal. 2009; 2(100):ra81. [PubMed: 19996457]
32. Freund A, Orjalo AV, Desprez PY, Campisi J. Inflammatory networks during cellular senescence: causes and consequences. Trends Mol. Med. 2010; 16(5):238–246. [PubMed: 20444648]
33. Voghel G, Thorin-Trescases N, Farhat N, et al. Cellular senescence in endothelial cells from atherosclerotic patients is accelerated by oxidative stress associated with cardiovascular risk factors. Mech. Ageing Dev. 2007; 128(11–12):662–671. [PubMed: 18022214]
34. Zhao T, Li J, Chen AF. MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. Am. J. Physiol. Endocrinol. Metab. 2010; 299(1):E110–E116. [PubMed: 20424141]

35. Potente M, Dimmeler S. Emerging roles of SIRT1 in vascular endothelial homeostasis. Cell Cycle. 2008; 7(14):2117–2122. [PubMed: 18641460]
36. Potente M, Ghaeni L, Baldessari D, et al. SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev. 2007; 21(20):2644–2658. [PubMed: 17938244]
37. Menghini R, Casagrande V, Cardellini M, et al. MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation. 2009; 120(15):1524–1532. [PubMed: 19786632] • Describes miR-217 as an endogenous inhibitor of SirT1, which promotes endothelial senescence.
38. Poliseno L, Tuccoli A, Mariani L, et al. MicroRNAs modulate the angiogenic properties of HUVECs. Blood. 2006; 108(9):3068–3071. [PubMed: 16849646]
39. Suarez Y, Fernandez-Hernando C, Yu J, et al. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc. Natl Acad. Sci. USA. 2008; 105(37):14082–14087. [PubMed: 18779589]
40. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ. Res. 2007; 101(1):59–68. [PubMed: 17540974]
41. Chen Y, Banda M, Speyer CL, et al. Regulation of the expression and activity of the antiangiogenic homeobox gene *GAX/MEOX2* by ZEB2 and microRNA-221. Mol. Cell. Biol. 2010; 30(15):3902–3913. [PubMed: 20516212]
42. Anand S, Majeti BK, Acevedo LM, et al. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. Nat. Med. 2010; 16(8):909–914. [PubMed: 20676106]
43. Wang S, Aurora AB, Johnson BA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell. 2008; 15(2):261–271. [PubMed: 18694565]
44. Fish JE, Santoro MM, Morton SU, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev. Cell. 2008; 15(2):272–284. [PubMed: 18694566]
45. Suarez Y, Sessa WC. MicroRNAs as novel regulators of angiogenesis. Circ. Res. 2009; 104(4): 442–454. [PubMed: 19246688] •• Describes the approaches and current experimental evidence for the involvement of miRNAs in the angiogenic process; potential therapeutic applications for vascular diseases associated with abnormal angiogenesis are also addressed.
46. Zhang C. MicroRNAs in vascular biology and vascular disease. J. Cardiovasc. Transl Res. 2010; 3(3):235–240. [PubMed: 20560045]
47. van Solingen C, Seghers L, Bijkerk R, et al. Antagomir-mediated silencing of endothelial cell specific microRNA-126 impairs ischemia-induced angiogenesis. J. Cell Mol. Med. 2009; 13(8A): 1577–1585. [PubMed: 19120690]
48. Bonauer A, Carmona G, Iwasaki M, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science. 2009; 324(5935):1710–1713. [PubMed: 19460962] •• Describes the expression of the miR-17–92 cluster in human endothelial cells and the role of miR-92a in angiogenesis.
49. Minami Y, Satoh M, Maesawa C, et al. Effect of atorvastatin on microRNA 221/222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. Eur. J. Clin. Invest. 2009; 39(5):359–367. [PubMed: 19371267]
50. Chen T, Huang Z, Wang L, et al. MicroRNA-125a-5p partly regulates the inflammatory response, lipid uptake, and ORP9 expression in oxLDL-stimulated monocyte/macrophages. Cardiovasc. Res. 2009; 83(1):131–139. [PubMed: 19377067]
51. Olkkonen VM, Levine TP. Oxysterol binding proteins: in more than one place at one time? Biochem. Cell Biol. 2004; 82(1):87–98. [PubMed: 15052330]
52. Raychaudhuri S, Im YJ, Hurley JH, Prinz WA. Nonvesicular sterol movement from plasma membrane to ER requires oxysterol-binding protein-related proteins and phosphoinositides. J. Cell Biol. 2006; 173(1):107–119. [PubMed: 16585271]
53. Fang Y, Shi C, Manduchi E, Civelek M, Davies PF. MicroRNA-10a regulation of proinflammatory phenotype in atherosusceptible endothelium *in vivo* and *in vitro*. Proc. Natl Acad. Sci. USA. 2010; 107(30):13450–13455. [PubMed: 20624982] •• Describes miR-10a, which targets HOX1 and is involved in unique signaling pathways, including the regulation of inflammation.

54. Boettger T, Beetz N, Kostin S, et al. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the miR143/145 gene cluster. J. Clin. Invest. 2009; 119(9):2634–2647. [PubMed: 19690389]
55. Kawai-Kowase K, Owens GK. Multiple repressor pathways contribute to phenotypic switching of vascular smooth muscle cells. Am. J. Physiol. Cell Physiol. 2007; 292(1):C59–C69. [PubMed: 16956962]
56. Parmacek MS. MicroRNA-modulated targeting of vascular smooth muscle cells. J. Clin. Invest. 2009; 119(9):2526–2528. [PubMed: 19690387]
57. Cordes KR, Sheehy NT, White MP, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature. 2009; 460(7256):705–710. [PubMed: 19578358] •• Describes miR-145 and miR-143 function in regulating the quiescent versus proliferative phenotype of vascular smooth muscle cells.
58. Elia L, Quintavalle M, Zhang J, et al. The knockout of miR-143 and -145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. Cell Death Differ. 2009; 16(12):1590–1598. [PubMed: 19816508]
59. Xin M, Small EM, Sutherland LB, et al. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev. 2009; 23(18):2166–2178. [PubMed: 19720868]
60. Quintavalle M, Elia L, Condorelli G, Courtneidge SA. MicroRNA control of podosome formation in vascular smooth muscle cells *in vivo* and *in vitro*. J. Cell Biol. 2010; 189(1):13–22. [PubMed: 20351064]
61. Liu X, Cheng Y, Zhang S, et al. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. Circ. Res. 2009; 104(4):476–487. [PubMed: 19150885] • Describes the roles of miR-221 and miR-222 in regulating vascular smooth muscle cell proliferation and neointimal hyperplasia.
62. Dietschy JM, Turley SD. Control of cholesterol turnover in the mouse. J. Biol. Chem. 2002; 277(6):3801–3804. [PubMed: 11733542]
63. Brown MS, Ye J, Goldstein JL. Medicine. HDL miR-ed down by SREBP introns. Science. 2010; 328(5985):1495–1496. [PubMed: 20558698]
64. Rayner KJ, Suarez Y, Davalos A, et al. MiR-33 contributes to the regulation of cholesterol homeostasis. Science. 2010; 328(5985):1570–1573. [PubMed: 20466885]
65. Najafi-Shoushtari SH, Kristo F, Li Y, et al. MicroRNA-33 and the *SREBP* host genes cooperate to control cholesterol homeostasis. Science. 2010; 328(5985):1566–1569. [PubMed: 20466882]
66. Elmen J, Lindow M, Schutz S, et al. LNA-mediated microRNA silencing in non-human primates. Nature. 2008; 452(7189):896–899. [PubMed: 18368051]
67. Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. Cell Metab. 2006; 3(2):87–98. [PubMed: 16459310]
68. Krutzfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs *in vivo* with ‘antagomirs’. Nature. 2005; 438(7068):685–689. [PubMed: 16258535] • Describes a novel class of chemically engineered oligonucleotides, termed ‘antago-miRNAs’.
69. Song H, Bu G. MicroRNA-205 inhibits tumor cell migration through downregulating the expression of the LDL receptor-related protein 1. Biochem. Biophys. Res. Commun. 2009; 388(2):400–405. [PubMed: 19665999]
70. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2003; 284(1):R1–R12. [PubMed: 12482742]
71. Davis ME, Grumbach IM, Fukai T, Cutchins A, Harrison DG. Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor κB binding. J. Biol. Chem. 2004; 279(1):163–168. [PubMed: 14570928]
72. Weber M, Baker MB, Moore JP, Searles CD. MiR-21 is induced in endothelial cells by shear stress and modulates apoptosis and eNOS activity. Biochem. Biophys. Res. Commun. 2010; 393(4):643–648. [PubMed: 20153722]
73. Chan LS, Yue PY, Mak NK, Wong RN. Role of microRNA-214 in ginsenoside-Rg1-induced angiogenesis. Eur. J. Pharm. Sci. 2009; 38(4):370–377. [PubMed: 19733659]

74. Dentelli P, Rosso A, Orso F, et al. microRNA-222 controls neovascularization by regulating signal transducer and activator of transcription 5A expression. Arterioscler. Thromb. Vasc. Biol. 2010; 30(8):1562–1568. [PubMed: 20489169]
75. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci. 2010; 101(10):2087–2092. [PubMed: 20624164]
76. Gilad S, Meiri E, Yogev Y, et al. Serum microRNAs are promising novel biomarkers. PLoS ONE. 2008; 3(9):e3148. [PubMed: 18773077]
77. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc. Natl Acad. Sci. USA. 2008; 105(30):10513–10518. [PubMed: 18663219]
78. Hunter MP, Ismail N, Zhang X, et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS ONE. 2008; 3(11):e3694. [PubMed: 19002258]
79. D'Alessandra Y, Devanna P, Limana F, et al. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. Eur. Heart J. 2010; 31(22):2765–2773. [PubMed: 20534597]
80. Guo M, Mao X, Ji Q, et al. miR-146a in PBMCs modulates Th1 function in patients with acute coronary syndrome. Immunol. Cell Biol. 2010; 88(5):555–564. [PubMed: 20195282]
81. Ai J, Zhang R, Li Y, et al. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. Biochem. Biophys. Res. Commun. 2010; 391(1):73–77. [PubMed: 19896465]
82. Cheng Y, Tan N, Yang J, et al. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. Clin. Sci. (Lond.). 2010; 119(2):87–95. [PubMed: 20218970]
83. Wang GK, Zhu JQ, Zhang JT, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. Eur. Heart J. 2010; 31(6):659–666. [PubMed: 20159880]
84. Adachi T, Nakanishi M, Otsuka Y, et al. Plasma microRNA 499 as a biomarker of acute myocardial infarction. Clin. Chem. 2010; 56(7):1183–1185. [PubMed: 20395621]
85. Ji X, Takahashi R, Hiura Y, et al. Plasma miR-208 as a biomarker of myocardial injury. Clin. Chem. 2009; 55(11):1944–1949. [PubMed: 19696117]
86. Fichtlscherer S, De Rosa S, Fox H, et al. Circulating microRNAs in patients with coronary artery disease. Circ. Res. 2010; 107(5):677–684. [PubMed: 20595655]
87. Tan KS, Armugam A, Sepramaniam S, et al. Expression profile of microRNAs in young stroke patients. PLoS ONE. 2009; 4(11):e7689. [PubMed: 19888324]
88. Lee SD, Shroyer KR, Markham NE, et al. Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. J. Clin. Invest. 1998; 101(5):927–934. [PubMed: 9486960]
89. Caruso P, MacLean MR, Khanin R, et al. Dynamic changes in lung microRNA profiles during the development of pulmonary hypertension due to chronic hypoxia and monocrotaline. Arterioscler. Thromb. Vasc. Biol. 2010; 30(4):716–723. [PubMed: 20110569]
90. Chen Z, Nakajima T, Tanabe N, et al. Susceptibility to chronic thromboembolic pulmonary hypertension may be conferred by miR-759 via its targeted interaction with polymorphic fibrinogen α gene. Hum. Genet. 2010; 128(4):443–452. [PubMed: 20677013]
91. Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. Ann. NY Acad. Sci. 2001; 936:11–30. [PubMed: 11460466]
92. Cheng Y, Zhang C. MicroRNA-21 in cardiovascular disease. J. Cardiovasc. Transl. Res. 2010; 3(3):251–255. [PubMed: 20560046]
93. Ji R, Cheng Y, Yue J, et al. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of microRNA in vascular neointimal lesion formation. Circ. Res. 2007; 100(11):1579–1588. [PubMed: 17478730] • Describes the expression and function of miR-21 in neointimal lesion formation.
94. Vendrov AE, Madamanchi NR, Hakim ZS, Rojas M, Runge MS. Thrombin and NAD(P)H oxidase-mediated regulation of CD44 and BMP4-Id pathway in VSMC, restenosis, and atherosclerosis. Circ. Res. 2006; 98(10):1254–1263. [PubMed: 16601225]
95. Lin Y, Liu X, Cheng Y, et al. Involvement of microRNAs in hydrogen peroxide-mediated gene regulation and cellular injury response in vascular smooth muscle cells. J. Biol. Chem. 2009; 284(12):7903–7913. [PubMed: 19158092]

96. Chan MC, Hilyard AC, Wu C, et al. Molecular basis for antagonism between PDGF and the TGFβ family of signalling pathways by control of miR-24 expression. EMBO J. 2010; 29(3):559–573. [PubMed: 20019669]

97. Moser M, Patterson C. Bone morphogenetic proteins and vascular differentiation: BMPing up vasculogenesis. Thromb. Haemost. 2005; 94(4):713–718. [PubMed: 16270622]

98. Fluiter K, Mook OR, Baas F. The therapeutic potential of LNA-modified siRNAs: reduction of off-target effects by chemical modification of the siRNA sequence. Methods Mol. Biol. 2009; 487:189–203. [PubMed: 19301648] • Describes the limitations and possibilities of locked nucleotide acid-modified siRNA and its possible use in therapeutic applications.

99. van Rooij E, Marshall WS, Olson EN. Toward microRNA-based therapeutics for heart disease: the sense in antisense. Circ. Res. 2008; 103(9):919–928. [PubMed: 18948630]

100. Xiao J, Yang B, Lin H, et al. Novel approaches for gene-specific interference via manipulating actions of microRNAs: examination on the pacemaker channel genes *HCN2* and *HCN4*. J. Cell. Physiol. 2007; 212(2):285–292. [PubMed: 17516552]