HHS Public Access

Author manuscript

*Curr Opin Hematol*. Author manuscript; available in PMC 2018 October 08.

Published in final edited form as:

*Curr Opin Hematol*. 2018 May ; 25(3): 227–236. doi:10.1097/MOH.0000000000000424.

# MicroRNAs in endothelial cell homeostasis and vascular disease

**Carlos Fernández-Hernando** and **Yajaira Suárez**

Program in Integrative Cell Signaling and Neurobiology of Metabolism, Department of Comparative Medicine, Department of Pathology and the Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, Connecticut, USA

## Abstract

**Purpose of review—**Since the first discovery of microRNAs (miRNAs) in 1993, the involvement of miRNAs in different aspects of vascular disease has emerged as an important research field. In this review, we summarize the fundamental roles of miRNAs in controlling endothelial cell functions and their implication with several aspects of vascular dysfunction.

**Recent findings—**MiRNAs have been found to be critical modulators of endothelial homeostasis. The dysregulation of miRNAs has been linked to endothelial dysfunction and the development and progression of vascular disease which and open new opportunities of using miRNAs as potential therapeutic targets for vascular disease.

**Summary—**Further determination of miRNA regulatory circuits and defining miRNAs-specific target genes remains key to future miRNA-based therapeutic applications toward vascular disease prevention. Many new and unanticipated roles of miRNAs in the control of endothelial functions will assist clinicians and researchers in developing potential therapeutic applications.

### Keywords

endothelial cells; microRNA therapeutics; micrornas; vascular disease

## INTRODUCTION

The vascular endothelium is a multifunctional organ and is critically involved in modulating vascular tone and structure. The endothelium is the monolayer of endothelial cells lining the lumen of blood vessels in every organ system [1]. Endothelial cells are specially designed and spatially located to detect changes in hemodynamic forces and blood-borne signals, facilitate the bidirectional passage of nutrient substances and active molecules from blood to tissues, and control the passage of blood cells themselves [2]. Endothelial cells are metabolically active with important paracrine, endocrine, and autocrine functions, indispensable for the maintenance of vascular homeostasis under physiological conditions [3–10]. Thus, the vascular endothelium is basically involved in the regulation of several aspects of vascular homeostasis, which includes: control of blood vessel development,

Correspondence to Yajaira Suárez, PhD, Yale University School of Medicine, 10 Amistad Street, Amistad Research Building 301-B, New Haven, CT 06520, USA. Tel: +1 203 737 8858; fax: +1 203 737 2290; yajaira.suarez@yale.edu.

Conflicts of interest

There are no conflicts of interest.

growth and differentiation; control of leukocyte trafficking; control of vascular tone; control of vascular barrier; control of platelet function, coagulation, and fibrinolysis [3–5,7–9,11].

Alterations in normal endothelial cell functions have been implicated in several diseases including atherosclerosis, diabetes, tumor metastasis, inflammatory diseases (e.g., rheumatoid arthritis), and hypertension [6,12–16]. Vascular dysfunction perturbs the balance between vasoconstriction and vasodilation and initiates a number of events that trigger endothelial cell activation and prompt the vessel wall to increased endothelial permeability, leukocyte adherence, endothelial proliferation, prooxidation, and thrombosis [6,8,12–22]. All the processes in which endothelial cells are involved require a fine-tune synchronization of a series of molecular and cellular events triggered by both stimulatory and inhibitory signals that converge into a physiological regulated response. As such, highly dynamic and dose-sensitive signaling complexes are prime candidates for microRNA (miRNA) posttranscriptional-mediated regulation of gene expression programs of endothelial cells [23–26].

In the present review, we summarize and discuss the role of miRNAs in regulating endothelial cell functions associated vascular pathophysiological conditions.

## REGULATION OF VASCULAR DEVELOPMENT, GROWTH AND DIFFERENTIATION

During adulthood, the endothelium remains essentially quiescent, to fulfill its main function in conducting nutritive blood flow to organs, with turnover rates on the orders of months to years. Rapid changes in endothelial cell proliferation rates occurs following activation of endothelium by angiogenic cytokines [27–31,32,33■]. In fact, in the healthy adult, angiogenesis occurs only in select phases of the female reproductive cycle, to allow physiological adipose tissue expansion, as a protection mechanism in wound healing/tissue repair, and is almost exclusively associated with disorder when angiogenesis is induced by microenvironmental factors such as hypoxia or inflammation [34–39]. The pathological processes associated with angiogenesis include diseases as diverse as cancer, macular degeneration, psoriasis, diabetic retinopathy, thrombosis, and inflammatory disorders, including arthritis and atherosclerosis. Moreover, insufficient angiogenesis is characteristic of ischemic heart disease, peripheral vascular disease, and preclampsia [34,40]. The first evidence of the importance of miRNAs in vascular neovascularization came from both in-vitro and invivo approaches to knockdown enzymes involved in the biogenesis of miRNAs [41–43] (Table 1).

microRNA (MiR)-126 is the prototype of an endothelial-specific miRNA, highly expressed in vascularized tissues, endothelial cells, and hematopoietic stem cells [43,47,81–83]. The role of miR-126 in vascular integrity and angiogenesis was reported by targeted deletion of miR-126 in mice [45,46] and its knockdown in zebrafish[47]. MiR-126 was shown to target sprouty-related protein 1 and phosphoinositol-3 kinase regulatory subunit 2 (p85-b), both negative regulators of the vascular endothelial growth factor (VEGF) pathway [45–47], enhanced angiopoietin-1 signaling through phosphoinositol-3 kinase regulatory subunit 2/ p85-b repression [48]. The miR-126/ epidermal growth factor-like protein 7 gene is

transcriptionally regulated by ETS proto-oncogene, transcription factor (Ets)-1 and Ets-2 in endothelial cells [81] and through the mechanosensitive transcription factor kruppel-like factor 2a (KLF2a) which in turn activated the VEGF pathway [49]. Consistent with the angiogenic properties of miR-126, van Solingen *et al.*[84] demonstrated that the use of antagomiR-126 impairs ischemia-induced angiogenesis.

The relationship between miRNAs and their host gene is especially relevant in miRNAs encoded within an angiogenic host gene including miR-149 [32] and the highly conserved miR-218 is an intronic miRNA encoded within the Slit2 and Slit3 genes [50–52]. Slits are secreted glycoproteins, which are the main ligands for roundabout receptors (Robos). In turn, miR-218 regulates Slit/Robo signaling through repression of Robo1, Robo2, and glucuronyl C5-epimerase, an enzyme involved in heparan sulfate proteoglycan bio-synthesis and thus regulating endothelial cell migration overall retinal vasculature [51] and required during heart development in zebrafish [50].

The miR-17–92 cluster is one of the best-characterized polycistronic miRNAs and is located in intron 3 of the C13orf25 gene (chromosome 13q31.3). MiR-17–92 encodes six individual miRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a, which are tightly grouped within an 800 base-pair region [85,86]. The miR-17–92 cluster is involved in cell proliferation, suppresses apoptosis of cancer cells, and induces tumor angiogenesis [87,88]. In the context of endothelial cells the miR-17–92 cluster is induced upon VEGF treatment [43]. In particular, extracellular-signal-regulated kinase/ETS transcription factor (Elk)1 activation is responsible for Elk-1-mediated transcription activation of the miR-17–92 cluster which in turn is necessary for endothelial cell proliferation and angiogenic sprouting [33■]. Interestingly, mice with conditional deletion of miR-17–92 have blunted physiological retinal angiogenesis during development and diminished VEGF-induced ear angiogenesis and tumor angiogenesis [33■]. The miR-106b-25 cluster, which is evolutionarily related to the miR-17–92 cluster, is also involved in angiogenesis, indeed its absence in a model of hindlimb ischemia impaired the capacity of restoring normal blood flow and significantly decreased capillary form[89], effect was attributed to the ability of the cluster to inhibit proliferation, migration, and viability in endothelial cells. In other model systems [53,54,90], members of the miR-17–92 cluster have different outcomes. Single overexpression of premiR-17, premiR-18a, premiR-19a, or premiR-20a significantly inhibits three-dimensional spheroid sprouting *in vitro*, whereas their individual inhibition has an opposite outcome[54]. This is in contrast to the genetic endothelial cell-specific deletion of the whole miR-17–92 cluster [33■]. A possible explanation for these discrepancies is that miR-17–92 levels vary significantly in response to VEGF, suggesting that growth and culture conditions influence basal miRNA expression, and therefore, cluster function in cultured endothelial cells. MiR-17–92 has been additionally involved in the regulation arterialization and highlights the importance of miRNA-mediated regulation of vascular wingless integrated protein signaling in maintaining arterial blood flow [55].

The miR-23–24–27 cluster is expressed in endothelial cells and vascularized tissues and is heavily involved in angiogenesis during vascular disorders and ischemic heart disease [56,57,91] [58]. In particular, miR-23 and miR-27 repress Sprouty2 and Semaphorin-6A, which negatively regulate Ras (rat sarcoma oncogene homologue derived protein)/mitogen-

activated protein kinase signaling and vascular endothelial growth factor receptor 2-mediated signaling, respectively [56] [59], and miR-24, is highly expressed in cardiac endothelial cells and its expression increases in cardiac ischemia and in hypoxic conditions [57] and targets the transcription factor [GATA2 (transcription factor that bind to “GATA” DNA sequence)] and the p21-activated kinase. Interestingly, the expression of several members of the miR-23–24–27 cluster, in particular miR-23b and miR-27, are upregulated by shear stress and correlate with pulsatile shear-induced endothelial cell growth arrest [92].

As described above, miRNAs do not only control the angiogenic response, but their expression may also be regulated by proangiogenic factors [32,33■]. MiR-132 expression levels are undetectable in quiescent endothelium, whereas it is highly expressed in endothelium in tumors and hemangiomas and VEGF and basic fibroblast growth factor (bFGF) increased its expression through cAMP response element-binding protein activation in endothelial cells [27].

Interestingly, several studies have demonstrated that interleukin 3 and bFGF are released by infiltrated T-lymphocytes in the atherosclerotic plaque, promoting neovascularization [28,29]. Interleukin 3 and bFGF negatively modulated the expression of miR-221/miR-222 in endothelial cells and signal transducer and activator of transcription 5A, a transcription factor that regulates the expression of genes involved in cell proliferation and migration, was identified as target for miR-222 [30].

MiR-150 is contained in monocyte-derived microvesicles, both from the monocytic cell line, THP1, and from human peripheral blood monocytes[60]. The miRNA can be transferred to endothelial cells and induce downregulation of c- myeloblastosis transcription activator factor (c-Myb), which is responsible for the increased migratory capabilities of endothelial cells. Moreover, patients with severe atherosclerosis had circulating microvesicles that were enriched in miR-150 compared with control patients, suggesting that their microvesicles might be either partially responsible for the vascularization of atherosclerotic plaques or might be a marker of it, as they have a higher monocyte infiltration in the lesion [60].

As before anticipated, the formation of new blood vessels is an essential component in pathologies such as ischemia, where interrupted blood flow deprives tissues of oxygen and nutrients necessary to maintain their normal functions. In response to ischemia a class of miRNAs, known as ‘hypoximiRs’, is characterized by the altered expression in response to hypoxia [93]. MiR-210 is an intronic miRNA contained within the sequence of the non-protein transcript MIR210HG (miR-210 host gene) located on chromosome 11 and its expression is induced in hypoxic conditions in a hypoxiainducible factor-1α-dependent manner, enhancing the development of capillary-like structures and VEGF-induced chemotaxis in endothelial cells [61], whereas improving cardiac function in a murine model of myocardial infarction[62]. Additionally, the expression of miR-210 is upregulated in atherosclerotic plaques providing a link between this miRNA and endothelial cell dysfunction in atherosclerosis [94]. Given the complex stimuli in hypoxia, the angiogenic function of certain miRNAs differs compared with normoxia [31]. For instance, hypoxia increases the expression of miR-424 by PU (hematopoietic transcription factor derived from Spleen Focus Forming Virus homologue onco-gene).1-dependent transactivation in

endothelial cells. MiR-424 was shown to target cullin 2, a scaffolding protein critical for the assembly of the ubiquitin ligase system, which stabilizes hypoxiainducible factor-a resulting in increased proliferation and migratory capabilities in endothelial cells [63].

Endothelial cell senescence plays a crucial role in the pathogenesis of aging-related diseases such as cardiovascular dysfunction and atherosclerosis [95]. MiR-217 negatively regulates the expression of silent information regulator 1, a Nicotinamide ade-nine dinucleotide$^{+}$ −dependent deacetylase, which prevented stress-induced senescence and mediated angiogenesis through deacetylation of the forkhead transcription factor (Forkhead Box Protein O1) [64]. On the other hand, aging, which is associated with an increase of reactive oxygen species and promotes senescence, decreases the expression of miR-146 in endothelial cells which is implicated miR-146 in the negative regulation of NOX4, the main endothelial isoform of the NADPH (reduced Nicotinamide ade-nine dinucleotide phosphate) oxidases complex [65]. Interestingly, diminished autophagic activity has a major role in several aging-related disorders. MiR-216a has been shown to be induced during endothelial aging and thus mediating the reduction of the expression of Beclin 1, leading to an indirect downregulation of autophagy related 5 [66].

## REGULATION OF BARRIER FUNCTION

The vascular endothelium lining the inner surface of blood vessels serves as the first interface for circulating blood components to interact with cells of the vascular wall and surrounding extravascular tissues. Endothelial cells form a monolayer, within the blood vessel, by which the cells are linked to each other by different types of adhesive structures or cell–cell junctions (i.e., tight junctions, adherens junctions, and gap junctions) and cell attachments to extracellular matrix and basement membrane [96]. This permits a selective barrier to form for the transport of molecules between blood and tissues [97,98]. Appropriate regulation of these events maintains a low and selective permeability to fluid and solutes under normal physiological conditions. Endothelial barrier dysfunction occurs during stimulation by inflammatory agents, pathogens, activated blood cells, or disease states [99]. The pathophysiology is characterized by excessive flux of plasma across the exchange microvessel wall into the surrounding tissues [100].

Hantaviruses infect human endothelial cells and cause two diseases marked by vascular permeability defects, hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome [101]. In this setting, miR-155, miR-320, miR-126, and miR-222 have been shown to regulate adherens junction disassembly, cell migration, and cell morphology, which contribute to changes in vascular permeability [102,103]. Upon VEGF stimulation, the expression of miR-125b has been shown to be transiently induced in endothelial cells which in turn down-regulates the translation of vascular endothelialcadherin messenger RNA (mRNA) [67] and thus affecting normal barrier function [67,101]. More recently, miR-147b have been described to regulate endothelial barrier function by targeting a disintegrin and metalloproteinase15 expression. This protein has been shown to be upregulated and mediate endothelial hyperpermeability during inflammation and sepsis and in lipopolysaccharide-induced endothelial barrier dysfunction overexpression of miR-147b was attenuated this response [68] (Table 1).

## REGULATION OF VASCULAR TONE

Vascular tone is maintained by the endothelial cell-mediated release of numerous dilator, such as Nitric Oxide and prostacyclin or prostagladin I2, and constrictor substances including endothelin and platelet-activating factor [5]. These potent short-lived mediators that influence vascular hemodynamincs in the physiological state and, therefore, contribute to the regulation of blood pressure and blood flow. Nitric Oxide is generated in endothelial cells by the oxidation of L-Arginine to L-cytruline by endothelial nitric oxide synthase (eNOS) [104,105] which is a constitutively expressed gene in endothelial cells that can be upregulated by increases in shear stress of growth factors, such as VEGF and some drugs, including 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins). Conversely, hypoxia, lipopolysaccharide, tumor necrosis factor (TNF), and ox-low density lipoprotein (LDL) decrease its expression [106]. Preliminary studies showed that knockdown of Dicer increased eNOS expression in endothelial cells [42] (Table 1). Transfection of miR-221 and miR-222 mimics partially reversed the increases of eNOS protein attributable to Dicer silencing, suggesting miR-221 and miR-222 were involved in an indirect control of eNOS expression [42]. The direct control of eNOS expression is mediated by miR-155 downregulated eNOS expression through decreasing eNOS mRNA stability by binding to its 3-untranslated region [69]. Interestingly, TNF increased miR-155 expression in endothelial cells [72] and knockdown of miR-155 prevented cytokine-induced downregulation of eNOS expression, reduction of NO production, and impairment of endothelium-dependent vascular relaxation [69]. Recently, guanosine triphosphate cyclohydrolase 1 (GCH1) has been reported to be targeted of miR-133a. GCH1 deficiency is critical for eNOS uncoupling in endothelial dysfunction. Ectopic expression of miR-133a in endothelial cells mediates endothelial dysfunction induced by multiple cardiovascuar disease (CVD) risk factors. Interestingly, statin upregulates GCH1 gene expression by inhibiting aberrant miR-133a expression to prevent endothelial dysfunction [70■■].

Local disturbances in blood flow have been show to trigger smooth muscle cell contraction, migration, and proliferation in addition to activate platelet and leukocyte activation and adhesion [5,107–109]. These undesired effects explain why diminished Nitric Oxide secretion in regions with disturbed flow, such as arterial branch points, are prone to develop atheromas [110]. Additionally, hemodynamics itself has a profound effect on miRNA expression, and differentially regulated miRNAs contribute to the regulation of shear stress-mediated transcriptional programs [95,111]. Several reports showed that shear stress induced the expression of miR-21 with contrasting effects. In particular, laminar flow increased miR-21 and reduced apoptosis and increased Nitric Oxide production by modulating the Phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein Kinase B Alpha pathway, overall ameliorating endothelial cell functions [112]. However, under oscillatory shear miR-21 expression is induced promoting endothelial inflammation peroxisome proliferators-activated receptor-a and activator protein-1-mediated mechanisms [113].

Endothelial cells also synthesize endothelin-1 (ET-1), one of the most potent vasoconstrictors which expression and secretion is stimulated by hypoxia, shear stress and ischemia [114]. MiR-125a/b has been involved in the regulation of the potent vasoconstrictor ET-1. Interestingly, its expression has been shown to be regulated by oxidized LDLs [71].

Owing to the relevance of ET-1 in many vascular diseases such as hypertension, atherosclerosis and stroke, targeting of miR-125a/b could provide an important therapeutic approach.

## CONTROL OF LEUKOCYTE TRAFFICKING AND INFLAMMATION

Inflammation is usually defined as the local recruitment and activation of leukocytes [11,18,115]. Inflammatory cytokines including interleukin 1 and TNF-α, are predominantly involved in the inflammatory activation of endothelial cells acquiring new capacities, therefore, controlling a multi-step process in which leukocytes first transiently tether to and roll on the endothelial cell surface, then adhere more strongly and migrate over the endothelium, and finally squeeze between endothelial cells to emigrate from the intravascular space to the extravascular sites of inflammation [116,117]. The interaction of leukocytes with endothelial cells is mediated by the release of P-selectin from Weibel–Palade bodies [118,119] and increased expression of adhesion molecules, such as E-selectin (E-SELE), vascular cell-adhesion molecule 1 (VCAM1) and intracellular adhesion molecule1 [115,120].

Several studies have shown that inflammatory inducible expression of miRNAs also contribute to the regulation of inflammatory response of endothelial cells [121] (Table 1). In addition to miR-155 (prototype of TNF-induced miRNA in different cell types), TNF induces the expression of miR-31 and miR-17–3p, among others. Interestingly, E-SELE and intracellular adhesion molecule1 were identified and validated as targets of the TNF-induced miRNAs, miR-31, and miR-17–3p, respectively [72]. Specific antagonism of these TNF-induced miRNAs increased neutrophil adhesion to cultured endothelial cells. MiR-126, a constitutively expressed, but endothelial cell-restricted miRNA, has also been involved in vascular inflammation by modulating VCAM-1 expression induced by TNF [73]. However, the expression of this miRNA was not affected by TNF treatment [72]. TNF also induces the expression of miR-181b which in turn regulates the expression of importin-α3 (karyopherin subunit alpha 4), a protein required for nuclear translocation of nuclear factor-κB (NF-kB) and thus reducing the expression of responsive genes, such as VCAM1 and SELE in endothelial cells *in vitro* and *in vivo* [74].

MiR-10a also negatively regulate the NF-kB pathway by targeting mitogen-activated kinase kinase 7 (MAP3K7) and β-transducin repeat containing gene (βTRC), two regulators of the proteosomal degradation of NFKB inhibitor alpha and p65 translocation in endothelial cells [75]. The expression of miR-10a is decreased in athero-susceptible arterial regions, whereas the expression of MAP3K7 and βTRC is upregulated, suggesting that the differential expression of miR-10a could contribute to the regulation of proinflammatory endothelial phenotypes in athero-susceptible regions [75]. Interestingly, it has been recently shown that endothelial cells secrete extra-cellular vesicles [76] with potent antiinflammatory activities that can be attributable in part to the transfer of miR-10a to monocytes/macrophages and suppression of several components of the NF-kB pathway, including Interleukin-1 receptor-associated kinase 4 (IRAK4), β-TRC, and MAP3K7 [77■].

In addition to TNFα, interleukin 1β induce the expression of miR-146a and miR-146b in endothelial cells. MiR-146a/b upregulation favors the downregulation of adhesion molecules and proinflammatory genes, by suppressing NF-kB and MAP kinase/early growth response pathways by direct targeting of TNF receptor-associated factor 6 and IRAK1 [78]. In addition to its direct effect on inflammatory pathways, miR-146a targets human antigen R and, loss of this protein decreases cell adhesion molecules and inhibits endothelial activation by releasing the suppression on eNOS, probably by stabilization of the transcription factor KLF2 [78].

Atherosclerotic lesions preferentially originate and develop at arterial sites of curvatures, branches, and bifurcations where complex hemodynamic conditions of disturbed flow are associated with endothelial phenotypes expressing proinflammatory and procoagulant susceptibility [122]. Deficiency of Dicer in the endothelium of *Apolipoprotein E*$^{-/-}$ micereduced monocyte adhesion to the early atherosclerotic endothelium by downregulating chemokine (CX-C motif) ligand 1, and thereby diminished lesion formation. Thus, endothelial Dicer activity at arterial sites predisposed to atherosclerosis may play a proatherogenic role by generating proinflammatory miRNAs. This effect of Dicer is attributable to reduced endothelial miR-103 expression and the subsequent restoration of *KLF4* expression [123■].

Flow-sensitive miRNAs, known as ‘mechanomiRs’, modulate endothelial gene expression, and can regulate endothelial dysfunction and atherosclerosis. MiRNAs such as, miR-10a, 19a, 23b, 17 ~ 92, 21, 663, 92a, 143/145, 101, 126, 712, 205, and 155, have been identified as mechano-miRs [124]. Many of these miRNAs were initially identified as flow-sensitive *in vitro* and were later found to play a critical role in endothelial function and/or atherosclerosis *in vivo* through either gain-of-function or loss-of-function approaches. The key signaling pathways that are targeted by these mechanomiRs include the endothelial cell cycle, inflammation, apoptosis, and Nitric Oxide signaling [124]. On the other hand, Oxidized (Ox)-LDL which are accumulated within the atherosclerotic lesions, are known to enhance the expression of proinflamma-tory genes, leading to monocyte recruitment into the vessel wall and dysfunction of vascular endothelial cells [125]. Ox-LDL induces the downregulation of let-7 g in endothelial cells through the binding of octamer binding transcription factor-1 to the let-7 g promoter [126]. Interestingly, let-7 g, as well as let-7a and let-7b [79], target the lectin-like low-density lipoprotein receptor 1, which is the receptor for ox-LDL in endothelial cells. One of the most upregulated miRNAs in endothelial cells treated with ox-LDL is miR-365 and potentiates ox-LDL-mediated apoptosis by regulating the expression of Bcl-2 [80].

Endothelial cells can both produce extracellular vesicles that target other cells and modify their function [76], or they can be targets of extracellular vesicles derived from other cells. Human monocytes can secrete microvesicles containing miRNAs that are uptaken by endothelial cells and increase their migratory capabilities [127]. MiR-150 is contained in monocyte-derived microvesicles, both from the monocytic cell line, THP1, and from human peripheral blood monocytes [60]. The miRNA can be transferred to endothelial cells and induce down-regulation of c-Myb, which is responsible for the increased migratory capabilities of endothelial cells. Moreover, patients with severe atherosclerosis had

circulating microvesicles that were enriched in miR-150 compared with control patients, suggesting that their microvesicles might be either partially responsible for the vascularization of atherosclerotic plaques or might be a marker of it, as they have a higher monocyte infiltration in the lesion [60].

## CONCLUSION

MiRNAs are potential therapeutic targets as they possess biological potential to be used as strong biomarkers for the diagnosis of vascular diseases. Although the knowledge of how miRNAs affect endothelial functions has increased substantially within the past 10 years, we are still just beginning to understand the complex regulatory patterns of miRNAs, and many novel questions arise from the combinatorial effect of the different miRNAs. Additionally, miRNAs have also been recently shown to be differentially present in biofluids of patients with different vascular disease and shown to participate in cell–cell communication suggesting their wider functions in vascular diseases.

MiRNAs are critical in fine tuning and in maintaining the physiological balance of the vascular endothelium and appear as important targets for current miRNA-based therapies via reprogramming of endothelial cells using synthetic miRNA mimics or inhibitors. The effect of these novel therapeutic agents on several aspects of endothelial dysfunction remains to be properly assessed.

## Acknowledgements

The authors apologize to the many scientists whose contributions to the field could not be acknowledged owing to space limitations.

Financial support and sponsorship

Y.S. and C.F-H. laboratories are supported by grants from the National Institutes of Health (R01HL105945 and R01HL135012 to Y.S. and R35HL135820 to C.F-H.) and the American Heart Association (16GRNT26420047 to Y.S. and 16EIA27550005 to C.F-H.).

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■■ of outstanding interest

1. Fishman AP. Endothelium: a distributed organ of diverse capabilities. Ann N Y Acad Sci 1982; 401:1–8.
2. Cahill PA, Redmond EM. Vascular endothelium: gatekeeper of vessel health. Atherosclerosis 2016; 248:97–109. [PubMed: 26994427]
3. Arnout J, Hoylaerts MF, Lijnen HR. Haemostasis. Handb Exp Pharmacol 2006; 1–41.
4. Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. Physiol Rev 2004; 84:869–901. [PubMed: 15269339]
5. Busse R, Fleming I. Vascular endothelium and blood flow. Handb Exp Pharmacol 2006; 43–78.
6. Cines DB, Pollak ES, Buck CA, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 1998; 91:3527–3561. [PubMed: 9572988]

7. Hall AP. Review of the pericyte during angiogenesis and its role in cancer and diabetic retinopathy. Toxicol Pathol 2006; 34:763–775. [PubMed: 17162534]
8. Michiels C Endothelial cell functions. J Cell Physiol 2003; 196:430–443. [PubMed: 12891700]
9. Minshall RD, Malik AB. Transport across the endothelium: regulation of endothelial permeability. Handb Exp Pharmacol 2006; 107–144. [PubMed: 16999218]
10. Moncada S, Higgs EA. Nitric oxide and the vascular endothelium. Handb Exp Pharmacol 2006; 213–254.
11. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol 2007; 7:803–815. [PubMed: 17893694]
12. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. Int J Vasc Med 2012; 2012:918267. [PubMed: 22611498]
13. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004; 109:III27–III32. [PubMed: 15198963]
14. Sumpio BE, Riley JT, Dardik A. Cells in focus: endothelial cell. Int J Biochem Cell Biol 2002; 34:1508–1512. [PubMed: 12379270]
15. Goligorsky MS. Endothelial cell dysfunction: can't live with it, how to live without it. Am J Physiol Renal Physiol 2005; 288:F871–F880. [PubMed: 15821252]
16. O'Riordan E, Chen J, Brodsky SV, et al. Endothelial cell dysfunction: the syndrome in making. Kidney Int 2005; 67:1654–1658. [PubMed: 15840005]
17. Pearson JD. Normal endothelial cell function. Lupus 2000; 9:183–188. [PubMed: 10805485]
18. Pober JS, Cotran RS. The role of endothelial cells in inflammation. Transplantation 1990; 50:537–544. [PubMed: 2219269]
19. Verma S, Buchanan MR, Anderson TJ. Endothelial function testing as a biomarker of vascular disease. Circulation 2003; 108:2054–2059. [PubMed: 14581384]
20. Gimbrone MA, Jr, Garcia-Cardena G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. Cardiovasc Pathol 2013; 22:9–15. [PubMed: 22818581]
21. Folkman J Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995; 1:27–31. [PubMed: 7584949]
22. Lerman A, Zeiher AM. Endothelial function: cardiac events. Circulation 2005; 111:363–368. [PubMed: 15668353]
23. Ambros V The functions of animal microRNAs. Nature 2004; 431:350–355. [PubMed: 15372042]
24. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215–233. [PubMed: 19167326]
25. Ambros V MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. Cell 2003; 113:673–676. [PubMed: 12809598]
26. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of posttranscriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008; 9:102–114. [PubMed: 18197166]
27. Anand S, Majeti BK, Acevedo LM, et al. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. Nat Med 2010; 16:909–914. [PubMed: 20676106]
28. Dentelli P, Del Sorbo L, Rosso A, et al. Human IL-3 stimulates endothelial cell motility and promotes in vivo new vessel formation. J Immunol 1999; 163:2151–2159. [PubMed: 10438956]
29. Brogi E, Winkles JA, Underwood R, et al. Distinct patterns of expression of fibroblast growth factors and their receptors in human atheroma and nonatherosclerotic arteries. Association of acidic FGF with plaque microvessels and macrophages. J Clin Invest 1993; 92:2408–2418. [PubMed: 7693761]
30. 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; 30:1562–1568. [PubMed: 20489169]
31. Chamorro-Jorganes A, Araldi E, Penalva LO, et al. MicroRNA-16 and microRNA-424 regulate cell-autonomous angiogenic functions in endothelial cells via targeting vascular endothelial growth

factor receptor-2 and fibro-blast growth factor receptor-1. Arterioscler Thromb Vasc Biol 2011; 31:2595–2606. [PubMed: 21885851]

32. Chamorro-Jorganes A, Araldi E, Rotllan N, et al. Autoregulation of glypican-1 by intronic microRNA-149 fine tunes the angiogenic response to FGF2 in human endothelial cells. J Cell Sci 2014; 127:1169–1178. [PubMed: 24463821]
33. Chamorro-Jorganes A, Lee MY, Araldi E, et al. VEGF-induced expression of miR-17–92 cluster in endothelial cells is mediated by ERK/ELK1 activation and regulates angiogenesis. Circ Res 2016; 118:38–47. [PubMed: 26472816] ■ The study shows that VEGF triggers a cascade of signaling events, including mitogen activated protein kinase activation and Elk-1 phosphorylation which ultimately increases expression of miR-17–92 cluster members and represses phosphatase and tensing homolog, thereby supporting (endothelial cell) angiogenic fucntions. Endothelial genetic inactivation for miR-17–92 leads reduced angiogenic responses *in vivo*.
34. Carmeliet P Angiogenesis in life, disease and medicine. Nature 2005; 438:932–936. [PubMed: 16355210]
35. Coultas L, Chawengsaksophak K, Rossant J. Endothelial cells and VEGF in vascular development. Nature 2005; 438:937–945. [PubMed: 16355211]
36. Ferrara N Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol Cell Physiol 2001; 280: C1358–C1366. [PubMed: 11350730]
37. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med 2003; 9:677–684. [PubMed: 12778166]
38. Romagnani P, Lasagni L, Annunziato F, et al. CXC chemokines: the regulatory link between inflammation and angiogenesis. Trends Immunol 2004; 25:201–209. [PubMed: 15039047]
39. Yancopoulos GD, Davis S, Gale NW, et al. Vascular-specific growth factors and blood vessel formation. Nature 2000; 407:242–248. [PubMed: 11001067]
40. Carmeliet P Angiogenesis in health and disease. Nat Med 2003; 9:653–660. [PubMed: 12778163]
41. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of dicer and drosha for endothelial microRNA expression and angiogenesis. Circ Res 2007; 101:59–68. [PubMed: 17540974]
42. Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ Res 2007; 100:1164–1173. [PubMed: 17379831]
43. Suarez Y, Fernandez-Hernando C, Yu J, et al. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc Natl Acad Sci U S A 2008; 105:14082–14087. [PubMed: 18779589]
44. Poliseno L, Tuccoli A, Mariani L, et al. MicroRNAs modulate the angiogenic properties of HUVECs. Blood 2006; 108:3068–3071. [PubMed: 16849646]
45. Kuhnert F, Mancuso MR, Hampton J, et al. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. Development 2008; 135:3989–3993. [PubMed: 18987025]
46. Wang S, Aurora AB, Johnson BA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev cell 2008; 15:261–271. [PubMed: 18694565]
47. Fish JE, Santoro MM, Morton SU, et al. MiR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 2008; 15:272–284. [PubMed: 18694566]
48. Sessa R, Seano G, di Blasio L, et al. The miR-126 regulates angiopoietin-1 signaling and vessel maturation by targeting p85beta. Biochim Biophys Acta 2012; 1823:1925–1935. [PubMed: 22867989]
49. Nicoli S, Standley C, Walker P, et al. MicroRNA-mediated integration of haemodynamics and Vegf signalling during angiogenesis. Nature 2010; 464:1196–1200. [PubMed: 20364122]
50. Fish JE, Wythe JD, Xiao T, et al. A Slit/miR-218/Robo regulatory loop is required during heart tube formation in zebrafish. Development 2011; 138:1409–1419. [PubMed: 21385766]
51. Small EM, Sutherland LB, Rajagopalan KN, et al. MicroRNA-218 regulates vascular patterning by modulation of Slit-Robo signaling. Circ Res 2010; 107:1336–1344. [PubMed: 20947829]
52. Tie J, Pan Y, Zhao L, et al. MiR-218 inhibits invasion and metastasis of gastric cancer by targeting the Robo1 receptor. PLoS Genet 2010; 6:e1000879. [PubMed: 20300657]

53. Bonauer A, Carmona G, Iwasaki M, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 2009; 324:1710–1713. [PubMed: 19460962]
54. Doebele C, Bonauer A, Fischer A, et al. Members of the microRNA-17–92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood 2010; 115:4944–4950. [PubMed: 20299512]
55. Landskroner-Eiger S, Qiu C, Perrotta P, et al. Endothelial miR-17 approximately 92 cluster negatively regulates arteriogenesis via miRNA-19 repression of WNT signaling. Proc Natl Acad Sci U S A 2015; 112: 12812–12817. [PubMed: 26417068]
56. Zhou Q, Gallagher R, Ufret-Vincenty R, et al. Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23 27 24 clusters. Proc Natl Acad Sci U S A 2011; 108:8287–8292. [PubMed: 21536891]
57. Fiedler J, Jazbutyte V, Kirchmaier BC, et al. MicroRNA-24 regulates vascularity after myocardial infarction. Circulation 2011; 124:720–730. [PubMed: 21788589]
58. Bang C, Fiedler J, Thum T. Cardiovascular importance of the microRNA-23/27/24 family. Microcirculation 2012; 19:208–214. [PubMed: 22136461]
59. Urbich C, Kaluza D, Fromel T, et al. MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A. Blood 2012; 119:1607–1616. [PubMed: 22184411]
60. Zhang Y, Liu D, Chen X, et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol Cell 2010; 39:133–144. [PubMed: 20603081]
61. Fasanaro P, D'Alessandra Y, Di Stefano V, et al. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem 2008; 283:15878–15883. [PubMed: 18417479]
62. Hu S, Huang M, Li Z, et al. MicroRNA-210 as a novel therapy for treatment of ischemic heart disease. Circulation 2010; 122:S124–S131. [PubMed: 20837903]
63. Ghosh G, Subramanian IV, Adhikari N, et al. Hypoxia-induced microRNA-424 expression in human endothelial cells regulates HIF-alpha isoforms and promotes angiogenesis. J Clin Invest 2010; 120:4141–4154. [PubMed: 20972335]
64. Menghini R, Casagrande V, Cardellini M, et al. MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation 2009; 120:1524–1532. [PubMed: 19786632]
65. Vasa-Nicotera M, Chen H, Tucci P, et al. MiR-146a is modulated in human endothelial cell with aging. Atherosclerosis 2011; 217:326–330. [PubMed: 21511256]
66. Menghini R, Casagrande V, Marino A, et al. MiR-216a: a link between endothelial dysfunction and autophagy. Cell Death Dis 2014; 5:e1029. [PubMed: 24481443]
67. Muramatsu F, Kidoya H, Naito H, et al. MicroRNA-125b inhibits tube formation of blood vessels through translational suppression of VE-cadherin. Oncogene 2013; 32:414–421. [PubMed: 22391569]
68. Chatterjee V, Beard RS, Jr, Reynolds JJ, et al. MicroRNA-147b regulates vascular endothelial barrier function by targeting ADAM15 expression. PLoS One 2014; 9:e110286. [PubMed: 25333931]
69. Sun HX, Zeng DY, Li RT, et al. Essential role of microRNA-155 in regulating endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. Hypertension 2012; 60:1407–1414. [PubMed: 23108656]
70. Li P, Yin YL, Guo T, et al. Inhibition of aberrant microRNA-133a expression in endothelial cells by statin prevents endothelial dysfunction by targeting GTP cyclohydrolase 1 in vivo. Circulation 2016; 134:1752–1765. [PubMed: 27765794] ■■ The study uncovered a common mechanism by which multiple CVD risk factors induce endothelial dysfunction. In addition provides evidence that statins prevent the endothelial dysfunction by inhibit the aberrant expression of miR-133a in endothelial cells and upregulating GCH1 gene expression, resulting in restoration of BH4 and consequent eNOS recoupling.
71. Li D, Yang P, Xiong Q, et al. MicroRNA-125a/b-5p inhibits endothelin-1 expression in vascular endothelial cells. J Hypertens 2010; 28:1646–1654. [PubMed: 20531225]

72. Suarez Y, Wang C, Manes TD, Pober JS. Cutting edge: TNF-induced microRNAs regulate TNF-induced expression of E-selectin and intercellular adhesion molecule-1 on human endothelial cells: feedback control of inflammation. J Immunol 2010; 184:21–25. [PubMed: 19949084]
73. Harris TA, Yamakuchi M, Ferlito M, et al. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci U S A 2008; 105:1516–1521. [PubMed: 18227515]
74. Sun X, Icli B, Wara AK, et al. MicroRNA-181b regulates NF-kappaB-mediated vascular inflammation. J Clin Invest 2012; 122:1973–1990. [PubMed: 22622040]
75. Fang Y, Shi C, Manduchi E, et al. MicroRNA-10a regulation of proinflamma-tory phenotype in athero-susceptible endothelium in vivo and in vitro. Proc Natl Acad Sci U S A 2010; 107:13450–13455. [PubMed: 20624982]
76. Hergenreider E, Heydt S, Treguer K, et al. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol 2012; 14:249–256. [PubMed: 22327366]
77. Njock MS, Cheng HS, Dang LT, et al. Endothelial cells suppress monocyte activation through secretion of extracellular vesicles containing antiinflammatory microRNAs. Blood 2015; 125:3202–3212. [PubMed: 25838349] ■ The study showed that quiescent endothelial cells secrete extracellular vesicles that contain miR-10a can be taken up by monocytes to suppress the activation of the proinflammatory nuclear factor κB pathway.
78. Cheng HS, Sivachandran N, Lau A, et al. MicroRNA-146 represses endothelial activation by inhibiting pro-inflammatory pathways. EMBO Mol Med 2013; 5:1017–1034. [PubMed: 23733368]
79. Bao MH, Zhang YW, Lou XY, et al. Protective effects of let-7a and let-7b on oxidized low-density lipoprotein induced endothelial cell injuries. PLoS One 2014; 9:e106540. [PubMed: 25247304]
80. Qin B, Xiao B, Liang D, et al. MicroRNAs expression in ox-LDL treated HUVECs: MiR-365 modulates apoptosis and Bcl-2 expression. Biochem Biophys Res Commun 2011; 410:127–133. [PubMed: 21640710]
81. Harris TA, Yamakuchi M, Kondo M, et al. Ets-1 and Ets-2 regulate the expression of microRNA-126 in endothelial cells. Arterioscler Thromb Vasc Biol 2010; 30:1990–1997. [PubMed: 20671229]
82. Lagos-Quintana M, Rauhut R, Yalcin A, et al. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002; 12:735–739. [PubMed: 12007417]
83. Landgraf P, Rusu M, Sheridan R, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007; 129:1401–1414. [PubMed: 17604727]
84. 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; 13:1577–1585. [PubMed: 19120690]
85. Hayashita Y, Osada H, Tatematsu Y, et al. A polycistronic microRNA cluster, miR-17–92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005; 65:9628–9632. [PubMed: 16266980]
86. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. Nature 2005; 435:828–833. [PubMed: 15944707]
87. Dews M, Homayouni A, Yu D, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet 2006; 38:1060–1065. [PubMed: 16878133]
88. Otsuka M, Zheng M, Hayashi M, et al. Impaired microRNA processing causes corpus luteum insufficiency and infertility in mice. J Clin Invest 2008; 118:1944–1954. [PubMed: 18398510]
89. Semo J, Sharir R, Afek A, et al. The 106b 25 microRNA cluster is essential for neovascularization after hindlimb ischaemia in mice. Eur Heart J 2014; 35:3212–3223. [PubMed: 23420866]
90. Qin X, Wang X, Wang Y, et al. MicroRNA-19a mediates the suppressive effect of laminar flow on cyclin D1 expression in human umbilical vein endothelial cells. Proc Natl Acad Sci U S A 2010; 107:3240–3244. [PubMed: 20133739]
91. Chhabra R, Dubey R, Saini N. Cooperative and individualistic functions of the microRNAs in the miR-23a ~ 27a ~ 24–2 cluster and its implication in human diseases. Mol Cancer 2010; 9:232. [PubMed: 20815877]

92. Wang KC, Garmire LX, Young A, et al. Role of microRNA-23b in flow-regulation of Rb phosphorylation and endothelial cell growth. Proc Natl Acad Sci U S A 2010; 107:3234–3239. [PubMed: 20133741]

93. Greco S, Gaetano C, Martelli F. HypoxamiR regulation and function in ischemic cardiovascular diseases. Antioxid Redox Signal 2014; 21: 1202–1219. [PubMed: 24053126]

94. Raitoharju E, Lyytikainen LP, Levula M, et al. MiR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. Atherosclerosis 2011; 219:211–217. [PubMed: 21820659]

95. Schober A, Weber C. Mechanisms of MicroRNAs in Atherosclerosis. Annu Rev Pathol 2016; 11:583–616. [PubMed: 27193456]

96. Alexander JS, Elrod JW. Extracellular matrix, junctional integrity and matrix metalloproteinase interactions in endothelial permeability regulation. J Anat 2002; 200:561–574. [PubMed: 12162724]

97. Dejana E, Del Maschio A. Molecular organization and functional regulation of cell to cell junctions in the endothelium. Thromb Haemost 1995; 74:309–312. [PubMed: 8578477]

98. Schnittler HJ. Structural and functional aspects of intercellular junctions in vascular endothelium. Basic Res Cardiol 1998; 93(Suppl 3):30–39.

99. Dejana E, Corada M, Lampugnani MG. Endothelial cell-to-cell junctions. FASEB J 1995; 9:910–918. [PubMed: 7615160]

100. Baldwin AL, Thurston G. Mechanics of endothelial cell architecture and vascular permeability. Crit Rev Biomed Eng 2001; 29:247–278. [PubMed: 11417757]

101. Pepini T, Gorbunova EE, Gavrilovskaya IN, et al. Andes virus regulation of cellular microRNAs contributes to hantavirus-induced endothelial cell permeability. J Virol 2010; 84:11929–11936. [PubMed: 20844033]

102. Cichon C, Sabharwal H, Ruter C, Schmidt MA. MicroRNAs regulate tight junction proteins and modulate epithelial/endothelial barrier functions. Tissue Barriers 2014; 2:e944446. [PubMed: 25610754]

103. Nicoloso MS, Spizzo R, Shimizu M, et al. MicroRNAs–the micro steering wheel of tumour metastases. Nat Rev Cancer 2009; 9:293–302. [PubMed: 19262572]

104. Sessa WC. eNOS at a glance. J Cell Sci 2004; 117:2427–2429. [PubMed: 15159447]

105. Stamler JS, Singel DJ, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. Science 1992; 258:1898–1902. [PubMed: 1281928]

106. Govers R, Rabelink TJ. Cellular regulation of endothelial nitric oxide synthase. Am J Physiol Renal Physiol 2001; 280:F193–F206. [PubMed: 11208594]

107. Jeremy JY, Rowe D, Emsley AM, Newby AC. Nitric oxide and the proliferation of vascular smooth muscle cells. Cardiovasc Res 1999; 43:580–594. [PubMed: 10690330]

108. Lefer AM. Nitric oxide: nature's naturally occurring leukocyte inhibitor. Circulation 1997; 95:553–554. [PubMed: 9024134]

109. Mendelsohn ME, O'Neill S, George D, Loscalzo J. Inhibition of fibrinogen binding to human platelets by S-nitroso-N-acetylcysteine. J Biol Chem 1990; 265:19028–19034. [PubMed: 2172242]

110. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 2006; 6:508–519. [PubMed: 16778830]

111. Araldi E, Suarez Y. MicroRNAs as regulators of endothelial cell functions in cardiometabolic diseases. Biochim Biophys Acta 2016; 1861:2094–2103. [PubMed: 26825686]

112. 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; 393:643–648. [PubMed: 20153722]

113. Zhou J, Wang KC, Wu W, et al. MicroRNA-21 targets peroxisome proliferators-activated receptor-alpha in an autoregulatory loop to modulate flow-induced endothelial inflammation. Proc Natl Acad Sci U S A 2011; 108:10355–10360. [PubMed: 21636785]

114. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; 332: 411–415. [PubMed: 2451132]

115. Pober JS. Endothelial activation: intracellular signaling pathways. Arthritis Res 2002; 4(Suppl 3):S109–S116. [PubMed: 12110129]

116. Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 1991; 67:1033–1036. [PubMed: 1760836]

117. Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu Rev Physiol 1995; 57:827–872. [PubMed: 7778885]

118. Bonfanti R, Furie BC, Furie B, Wagner DD. PADGEM (GMP140) is a component of Weibel-Palade bodies of human endothelial cells. Blood 1989; 73:1109–1112. [PubMed: 2467701]

119. Middleton J, Neil S, Wintle J, et al. Transcytosis and surface presentation of IL-8 by venular endothelial cells. Cell 1997; 91:385–395. [PubMed: 9363947]

120. Bevilacqua MP, Nelson RM, Mannori G, Cecconi O. Endothelial-leukocyte adhesion molecules in human disease. Annu Rev Med 1994; 45:361–378. [PubMed: 7515220]

121. Sonkoly E, Pivarcsi A. Advances in microRNAs: implications for immunity and inflammatory diseases. J Cell Mol Med 2009; 13:24–38. [PubMed: 19175698]

122. Hajra L, Evans AI, Chen M, et al. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to athero-sclerotic lesion formation. Proc Natl Acad Sci U S A 2000; 97:9052–9057. [PubMed: 10922059]

123. Hartmann P, Zhou Z, Natarelli L, et al. Endothelial Dicer promotes athero-sclerosis and vascular inflammation by miRNA-103-mediated suppression of KLF4. Nat Commun 2016; 7:10521. [PubMed: 26837267] ■ The study suggests that Dicer promotes endothelial maladaptation and atherosclerosis in part by miR-103-mediated suppression of KLF4.

124. Kumar S, Kim CW, Simmons RD, Jo H. Role of flow-sensitive microRNAs in endothelial dysfunction and atherosclerosis: mechanosensitive athero-miRs. Arterioscler Thromb Vasc Biol 2014; 34:2206–2216. [PubMed: 25012134]

125. Li D, Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. Circulation 2000; 101:2889–2895. [PubMed: 10869259]

126. Chen KC, Hsieh IC, Hsi E, et al. Negative feedback regulation between microRNA let-7 g and the oxLDL receptor LOX-1. J Cell Sci 2011; 124:4115–4124. [PubMed: 22135361]

127. Nguyen MA, Karunakaran D, Geoffrion M, et al. Extracellular vesicles secreted by atherogenic macrophages transfer microrna to inhibit cell migration. Arterioscler Thromb Vasc Biol 2018; 38:49–63. [PubMed: 28882869]

## KEY POINTS

- MiRNAs initially discovered for their role in cell fate and differentiation decisions during organism development, in the past decade, have been demonstrated to be equally important in regulating the endothelial cell homeostasis.
- MiRNA-mediated gene regulation is involved in controlling every endothelia function and miRNA dysregulation results endothelial cell dysfunction and disruption normal endothelial cell homeostasis.
- Despite the highly complex nature of miRNA regulatory networks and the existence of technical limitations, recent advances have made miRNA biology a fascinating subject in vascular research and control of endothelial cell biology by miRNAs provides a powerful model to dissect the molecular orchestration of vascular function, homeostasis, growth, and differentiation.

**Table 1.**

Selected microRNAs involved in the regulation endothelial cell functions

| Function | miRNA | Target | Reference |
|---|---|---|---|
| Regulation of vascular development, growth, and differentiation | | | |
| | miR-126 | Spredl | [44–49] |
| | | PIK3R2/p85-b | |
| | miR-149 | Fgfrl | [32] |
| | miR-149* | Gpcl | |
| | miR-218 | Robo 1 | [50–52] |
| | | Robo2 | |
| | | Glce | |
| | miR-17~92 | Elkl | [33■,53–55] |
| | | Itga5 | |
| | | Jakl | |
| | | Fzd4 | |
| | miR-23–24–27 | Sprouty2 | [56–59] |
| | | Sema6A | |
| | | GATA2 | |
| | | PAK4 | |
| | miR-132 | p120RasGAP | [27] |
| | miR-221/222 | STAT5A | [30] |
| | miR-150 | c-Myb | [60] |
| | miR-210 | Efna3 | [61,62] |
| | | Ptplb | |
| | | Dapkl | |
| | | Ctgf | |
| | miR-424 | Cul2 | [63] |
| | miR-217 | Sirtl | [64] |
| | miR-146 | Nox4 | [65] |
| | miR-216a | Becnl | [66] |
| Regulation of barrier function | | | |

| Function | miRNA | Target | Reference |
|---|---|---|---|
| | miR-125 | Cadh5 | [67] |
| | miR-147b | Adaml5 | [68] |
| Regulation of vascular tone | | | |
| | miR-155 | eNOS | [69] |
| | miR-133a | Gchl | [70■■] |
| | miR-125a/b | Et-l | [71] |
| Control of leukocyte trafficking and inflammation | | | |
| | miR-31 | E-sele | [72] |
| | miR-17–3p | Icaml | [72] |
| | miR-126 | Vcaml | [73] |
| | miR-181b | Kpna4 | [74] |
| | miR-10a | Map3k7 | [75,76,77■] |
| | | βTRC | |
| | | IRAK4 | |
| | miR-146a/b | TRAF6 | [78] |
| | | IRAKl | |
| | | HuR | |
| | Let-7g/a/b | Loxl | [79] |
| | miR-365 | Bcl2 | [80] |
| | miR-150 | c-Myb | [60] |

ADAM, disintegrin and metalloproteinase domain-containing protein; ADAM15, a disintegrin and metalloproteinase15; c-Myb, c- myeloblastosis transcription activator factor; Cul2, cullin 2; E-sele, E-selectin; GCH, GTP cyclohydrolase 1; HuR, human antigen R; Icam1, intracellular adhesion molecule1; Glce, glucuronyl C5-epimerase; Lox, lectin-like oxidized low-density lipoprotein receptor; miRNA, microRNA; PIK3R2, phosphoinositol-3 kinase regulatory subunit 2; Robo, roundabout receptors; Sema6A, semaphorin-6A; Sirt1; silent information regulator 1; Spred1, sprouty-related protein1, STAT, signal transducer and activator of transcription.

*Passenger strand.