Abstract. The vascular system is essential for maintaining tissue homeostasis. Aberrant vascular remodeling underlies the pathogenesis and progression of many diseases, including cancer, atherosclerosis, coronary artery disease, and age-related macular degeneration. In response to various stimuli, neovascularization and neointima formation drive vascular pathogenesis in vascular disorders. Recent studies have shown microRNAs to be critical regulators of angiogenesis via modulation of multiple pathway components, some of which have been implicated in vascular disorders. Targeted microRNA-based therapeutics may hold promise in treating pathological angiogenesis, neointima formation, and other pathogenic processes in diverse vascular diseases.

Abbreviations

EC: endothelial cell; VSMC: vascular smooth muscle cells; CAD: coronary artery disease; AMD: age-related macular degeneration.
Introduction

The vascular system is the first functional organ system that develops in the vertebrate embryo, and it plays a fundamental role in development and tissue homeostasis in adulthood. Major defects in the developing vasculature often lead to early embryonic lethality, while aberrant vascular remodeling in adults is associated with numerous vascular diseases. During early development, a primitive vascular network forms by the differentiation of endothelial cells (ECs) from angioblast precursors via vasculogenesis. New vessels form from existing blood vessels by angiogenesis and subsequent remodeling. In response to angiogenic stimuli, ECs within blood vessels are activated to migrate and proliferate to form primary capillaries, which undergo remodeling through sprouting and intussusception. Further maturation of blood vessels into arteries and veins involves the incorporation of vascular smooth muscle cells (VSMCs) and deposition of extracellular matrix into the newly formed vessels. In adult tissues, most blood vessels remain quiescent and function to conduct nutritive blood flow. However, postnatal angiogenesis occurs in response to physiological and pathological events, such as reproduction, inflammation, tissue regeneration and tumor growth. Aberrant angiogenesis is associated with the pathogenesis of numerous diseases, including but not limited to cancer, atherosclerosis, ischemic heart disease, hypertension, diabetes, and age-related macular degeneration (AMD). Abnormal VSMC proliferation, migration and differentiation underlie neointimal lesion formation in the pathological processes of a variety of proliferative vascular diseases such as atherosclerosis, coronary heart disease and restenosis.

Recent studies have revealed important function for microRNAs (miRNAs or miRs) in cardiovascular disease and other disorders. miRNAs are small non-coding RNAs that repress gene expression post-transcriptionally, usually by targeting 3’-untranslated region (3’UTR) of mRNAs. Often, miRNAs modulate broad collections of mRNAs encoding multiple components of complex signaling pathways. Mounting evidence illustrates that miRNAs are important regulators of development and diseases, such as cardiovascular development and disease (for recent review, see [1]). Here we present a summary of recent progress describing the role of miRNAs in angiogenesis and vascular disease.

Requirement of miRNA pathway in angiogenesis

Similar to mRNAs, miRNAs are initially transcribed by RNA Polymerase II into primary miRNAs (pri-miRNA). Subsequent processing of
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pri-miRNAs into mature miRNA requires the sequential action of two RNase III endonucleases, Dicer and Drosha, respectively. The importance of miRNAs in angiogenesis was indicated by disrupting Dicer and Drosha function in vivo and in vitro. Mouse embryos with hypomorphic Dicer die at mid-gestation with defects in vascular remodeling [2]. Another independent Dicer hypomorphic mouse line shows female infertility with defective ovary angiogenesis [3]. Knockdown of Dicer in vitro in human ECs results in a decrease in Matrigel tube formation at baseline and in response to angiogenic factors [4-6]. Similarly, genetic silencing of Drosha also reduces EC sprouting and tube formation in vitro, although the effect is less profound [4]. The distinct difference between Dicer versus Drosha knockdown studies can be attributed to Drosha-independent miRNA biogenesis [7-8], or Dicer functions apart from miRNA maturation, such as maintaining heterochromatin or processing Alu RNAs [9-10]. Recently, EC-specific deletion of Dicer in mice provides direct in vivo evidence that endothelial miRNAs are required for postnatal angiogenesis response to angiogenic stimuli [11]. Knockout of Dicer in ECs using Tie2-Cre or Tamoxifen-inducible VECad-Cre reduced postnatal angiogenesis in response to a variety of stimuli, including exogenous VEGF, tumors, limb ischemia, and wound healing. Dicer silencing leads to upregulation of thrombospondin-1 (Tsp-1) [4, 11], a potent inhibitor of angiogenesis, as well as altered expression of other key regulators of endothelial biology and angiogenesis, such as TEK/Tie2, KDR/VEGFR2, Tie-1, eNOS and interleukin(IL)-8 [6]. Taken together, these studies illustrate the requirement of miRNA pathways in angiogenesis.

**Functional analysis of individual miRNAs in angiogenesis**

Emerging evidence has revealed an important function of individual miRNAs in angiogenesis (Fig. 1). The term “angiomiR” has been used to name miRNAs that regulate angiogenesis either cell autonomously or non-cell autonomously [12-13]. AngiomiRs are either expressed highly in ECs or regulated by angiogenic signals, such as hypoxia, serum and VEGF, and regulate angiogenesis by targeting multiple positive or negative regulators in angiogenic signaling pathways.

**miR-15/107 gene group**

The miR-15/107 group of miRNA genes includes miR-15a/b, miR-16, miR-103 and miR-107 in vertebrates [14]. In mammals, this group also includes miR-195, miR-424, miR-497, miR-503 and miR-646. These miRNAs are characterized by the identical AGCAGC sequence in the 5′-end
Figure 1. miRNAs involved in angiogenesis. The target proteins that mediate the angiogenic functions of the miRNAs are shown in grey. The proteins regulated by the target proteins are shown in black.

of the miRNAs, a “seed” region which is believed to be an important determinant of miRNA function.

Hypoxia stimulates angiogenesis by stabilizing hypoxia inducible factor (HIF)-1α and inducing VEGF expression. A specific spectrum of microRNAs, including miRs-23, -24, -26, -27, -181, -210, and -213, as well as miR-103 and miR-107 in miR-15/107 group, was shown to be induced in response to low oxygen [15]. The expression of miR-15, miR-16, and miR-20 was downregulated by hypoxia inducer DFOM [16]. miR-424, a mammalian member of miR-15/107 group, was differentially induced by hypoxia in ECs and SMCs, but not in tumor cells [17]. miR-424 expression is regulated by Ets family transcription factor PU.1, levels of which increase in nuclei of hypoxic endothelium. Similarly, the expression of rodent homolog of human miR-424, miR-322, is drastically upregulated under ischemic conditions in vivo in mouse models of coronary artery and femoral artery ligation. miR-424 stabilizes HIF-1α isoforms in ECs by targeting cullin 2, a scaffolding
protein essential for the assembly of the ubiquitin ligase system for HIF degradation (Fig. 1A). miR-424 promoted EC proliferation, migration and capillary tube formation in vitro and angiogenesis in vivo in the Matrigel, which was blocked by a specific morpholino oligonucleotide.

Overexpression of miR-15b or miR-16 repressed expression of target protein VEGF, while the opposite was observed upon knockdown of these miRNAs. Interestingly, miR-15a and miR-16-1 were frequently deleted or downregulated in the majority of chronic lymphocytic leukemia patients [18]. Also, overexpression of miR-15 and miR-16 induced apoptosis by targeting anti-apoptotic protein BCL2. These studies suggest that restoring miR-15 and miR-16 expression might be a promising strategy for tumor therapy by repressing tumor angiogenesis and inducing tumor cell death though targeting VEGF and BCL2 (Fig. 1A).

miR-107 was also shown to be critical in tumorigenesis. miR-107 expression was inversely associated with expression of its target protein HIF-1β in human colon cancer specimens [19]. Overexpression of miR-107 in tumor cells suppressed tumor angiogenesis, tumor growth and tumor VEGF expression in mice (Fig. 1A). Overall, these studies indicate that members of miR-15/107 gene group can mediate confounding regulation of angiogenic signaling in response to hypoxia/ischemia.

**miR-17~92 cluster**

The miR-17~92 cluster, also named Oncomir-1, is the first identified tumor promoting miRNA. This cluster contains miR-17, miR-18, miR-19a, miR-19b-1, miR-20a and miR-92-1. Two paralogs of miR-17~92, miR-106a~363 and miR-106b~25, also exist in mammals. miR-17~92 is frequently amplified and overexpressed in B cell lymphomas and lung cancers [20-22]. Additionally, miR-17~92 has been shown to be downstream of c-Myc and cooperate with c-Myc to induce B cell lymphoma in mice [23-24]. Overexpression of miR-17~92 in RAS expressing cells promotes tumor angiogenesis in vivo in a non-cell autonomous manner [25]. miR-17~92 promotes tumor angiogenesis by repressing anti-angiogenic target proteins thrombospondin-1 (Tsp1) and connective tissue growth factor (CTGF), as well as blunting TGFβ signaling pathway through targeting TGFβ receptor II (Fig. 1B) [25-26].

Members of the miR-17~92 cluster were also shown to repress angiogenesis, depending on the cellular context. The miR-17~92 cluster, along with miR-126, the miRNA-23~27~24 cluster, miR-221/222, miR-21 and the let-7 family, is among those highly expressed in ECs [4, 6, 27-29]. miR-17~92 members, including miR-17, -18a, -19a, 20a, and -92a, are
negative regulators of angiogenesis in ECs in vitro and in vivo [30-31]. Overexpression of these miRNAs inhibited EC sprouting in a 3-dimensional spheroid model, while inhibition of these miRNAs enhanced spheroid sprouting. Moreover, inhibition of miR-17/20 by specific cholesterol-conjugated anti-miRs (antagomiRs) selectively enhanced neovascularization of the Matrigel plugs but did not affect tumor angiogenesis. Among miR-17~92 cluster members, miR-92a is specifically upregulated by ischemia in a hindlimb ischemia model and an acute myocardial infarction model. Strikingly, in vivo knockdown of miR-92a resulted in improved recovery from ischemic damage due to accelerated vessel growth. Integrin α5 (ITG5) was validated as a proangiogenic factor responsible for the anti-angiogenic function of miR-92a, while the protein kinase Jak1 was identified as a proangiogenic molecule and a direct target for miR-17 (Fig. 1B). The opposing effects of miR-17~92 in ECs and tumor cells highlight the importance of cell type and cellular context on miRNA function. Deletion of the miR-106a-363 and miR-106b-25 clusters, either alone or in combination, did not result in any obvious phenotype. In contrast, mice with global deletion of miR-17~92 exhibit reduced birth weight and immediate perinatal fatality, likely due to severely hypoplastic lungs and ventricular septal defects [32]. To dissect the precise function of this cluster in vivo, a cell-type specific deletion of this cluster is warranted.

miR-93, one of the miRNAs within the miR-106b~25 cluster, also functions as an oncomir by enhancing tumor cell survival, blood cell expansion and tumor growth. The effects of miR-93 on tumor growth are at least partially mediated by its target gene integrinβ8, which can facilitate cell death and inhibit cell proliferation. Separately, miR-93 was shown to be downregulated in kidney cells under hyperglycemic conditions and in the glomeruli of db/db type 2 diabetic mice. Glomerular expression of miR-93 target protein VEGF was markedly increased upon miR-93 knockdown or knockout mice. VEGF plays a crucial role in the microvascular complications of diabetes including diabetic nephropathy. Identification of miR-93 as a novel regulator of VEGF in the diabetic setting could have important implications in the prevention of the progression of diabetic nephropathy. Again these studies illustrate the cellular context dependent function of miRNAs.

miR-21

miR-21 is known to promote tumor growth and metastasis [33-35]. When overexpressed in tumor cells, miR-21 promotes tumor angiogenesis through activation of AKT and ERK1/2 signaling pathways and enhancing HIF-1α expression (Fig. 1C) [36]. In ECs, however, miR-21 acts as a negative
regulator of angiogenesis. miR-21 overexpression reduced EC proliferation, migration and tube formation in the Matrigel, whereas inhibition of miR-21 using specific locked nucleic acid (LNA)-modified anti-miR led to opposite effects [37]. The anti-angiogenic action of miR-21 can be attributed to the repression of RhoGTPase RhoB, which functions as a modulator of EC migration and proliferation (Fig. 1C). Choroidal neovascularization (CNV), which involves abnormal growth of blood vessels in the back of the eye, is a hallmark of neovascular age-related macular degeneration (AMD) [38]. Intravitreal injection of miR-21 precursor reduced pathological angiogenesis to approximately fifty percent in a laser-induced CNV mouse model, suggesting therapeutic potential for miR-21 in vascular diseases with pathological angiogenesis. However, homozygous miR-21 knock-out mice are viable, fertile, and without any gross phenotypic differences compared to wild-type littermates, suggesting miR-21 is not required for developmental angiogenesis [39].

**miR-23~27~24 clusters**

Two miR-23~27~24 clusters exist in the vertebrate genome: an intergenic miR-23a~27a~24-2 cluster and an intronic miR-23b~27b~24-1 cluster. The miR-23a~27a~24-2 cluster encodes a pri-miRNA transcript composed of three miRNAs: miR-23a, miR-27a and miR-24-2, while the miR-23b~27b~24-1 cluster encodes a pri-miRNA transcript containing miR-23b, miR-27b and miR-24-1. The mature miRNA sequences of miR-23a/b, miR-27a/b and miR-24 are conserved among vertebrate species. miR-23a and miR-27a differ by only one nucleotide near their 3’ ends compared to their paralogs miR-23b and miR-27b, while the sequence of miR-24-1 and miR-24-2 is the same. Among these clusters, miR-27b has been considered to be pro-angiogenic based on the evidence that an inhibitor against this miRNAs reduced sprouting angiogenesis *in vitro* [4]. Research in our lab extends those findings to demonstrate that miR-23 and miR-27 are required for proper angiogenesis *in vitro* and *in vivo* [40]. Inhibition of miR-23 and miR-27 function by locked nucleic acid (LNA) modified anti-miRNAs repressed EC proliferation, migration and vascular network formation on Matrigel *in vitro* and postnatal retinal vascular development *in vivo*. Moreover, inhibition of miR-23 and miR-27 repressed pathological angiogenesis by about fifty percent in a laser-induced choroidal neovascularization mouse model. miR-23 and miR-27 enhance angiogenesis by promoting angiogenic signaling through targeting Sprouty2 and Semaphorin (Sema)-6A proteins (Fig. 1D). Sprouty proteins function as intracellular inhibitors of the MAP kinase pathway by mediating interference
of phosphorylation and therefore the activation of Raf, an upstream activator of the MAP kinase pathway. Sema6A has also been reported to repress angiogenic signaling [41]. We found that SEMA6A significantly repressed the phosphorylation of VEGFR2 and ERK1/2 in response to VEGF. Sprouty2 knockdown rescued the sprouting defects caused by miR-23/27 silencing, indicating that Sprouty2 plays a major role in mediating miR-23/27 angiogenic effects. The identification of Sprouty2 as target for both miR-23 and miR-27 is consistent with a recent report that Sprouty2 is a target for miR-27a during cancer cell growth and migration [42]. Our results suggest that therapeutic manipulation of miR-23/27 represents a potential strategy in treating CNV in patients with neovascular AMD and other vascular diseases. The identification of miR-23 and miR-27 as important regulators of MAPK activation also suggests roles for these miRNAs in cancer. The potential role of miR-24 in angiogenesis awaits future studies.

**miR-100**

miR-100 was identified as an anti-angiomiR in an effort to identify miRNAs involved in adaptive vessel growth following arterial occlusion [43]. miR-100 was significantly downregulated in the ischemic limb. *In vitro* evidence indicates that miR-100 modulates proliferation, tube formation and sprouting activity of ECs and migration of VSMCs. miR-100 functions as an endogenous repressor of the serine/threonine protein kinase mammalian target of rapamycin (mTOR), which has been shown to enhance angiogenic signaling in ECs (Fig. 1E). Overexpression of an mTOR construct lacking the miRNA binding site rescued the inhibitory effect of miR-100 on cell proliferation. Furthermore, inhibition of miR-100 by specific antagomiRs *in vivo* stimulated angiogenesis and improved functional perfusion after femoral artery occlusion in mice, an effect which was reversed by the mTOR inhibitor rapamycin. These studies establish miR-100 as an anti-angiogenic miRNA and an endogenous repressor of mTOR signaling, with potential implications in cardiovascular and neoplastic diseases.

**miR-126**

miR-126 is the best characterized pro-angiogenic miRNA and is required for maintaining vascular integrity and angiogenesis [44-46]. In both mouse and zebrafish, miR-126 is enriched in highly vascularized tissues, including the lung and heart. The expression of miR-126 appears to be restricted to EC and hematopoietic cell lineages [44, 47]. The EC-specific expression of miR-126 is driven by two regions in the 5.4kb 5’ flanking DNA upstream of
miR-126, which contains multiple binding sites for Ets transcription factors [44, 48]. In mammals, miR-126 is encoded in the intron 7 of EGF-like domain 7 (Egfl7) gene, which encodes an EC-specific secretory peptide that functions as a chemoattractant and inhibitor of smooth muscle cell migration. Incorporation of a miRNA into an intron of a tissue specific gene provides an efficient mechanism for coregulation of the miRNA and its host gene program. Loss-of-function studies in mice and zebrafish revealed an important function of miR-126 in governing vascular integrity and angiogenesis [44-46]. Targeted deletion of miR-126 in mice caused leaky vessels, hemorrhaging, and partial embryonic or perinatal lethality (40% of miR-126<sup>+/−</sup> mice), due to a loss of vascular integrity and defective angiogenesis. miR-126<sup>−/−</sup> mice showed severely delayed vascularization during cranial vessel and retinal vascular development. Furthermore, miR-126<sup>−/−</sup> ECs are defective in angiogenesis in response to angiogenesis factors, as shown by aortic ring assay in vitro, and Matrigel assay and corneal micropocket assay in vivo [22,43]. In zebrafish, knockdown of miR-126 resulted in hemorrhage and loss of vascular integrity during embryonic development [46], indicating a conserved function of miR-126.

Intriguingly, miR-126<sup>−/−</sup> mice display similar vascular abnormalities to the Egfl7<sup>/−</sup> mice reported previously, including edema, defective cranial vessel and retinal vascularization [44, 49]. This raised a controversy as to which molecule is responsible for the observed phenotype. In miR-126<sup>−/−</sup> mice, Egfl7 splicing and expression level is not changed, while miR-126 expression level in the Egfl7<sup>/−</sup> mice was not reported. Shortly after our report of the miR-126<sup>−/−</sup> mouse phenotype, floxed alleles of Egfl7 (Egfl7<sup>Δ/Δ</sup>) and miR-126 (miR-126<sup>Δ/Δ</sup>) were generated [50]. Egfl7<sup>Δ/Δ</sup> mice, in which miR-126 is not affected, are phenotypically normal. However, miR-126<sup>Δ/Δ</sup> mice, in which Egfl7 is normally expressed, recapitulated numerous embryonic and postnatal vascular phenotypes in the previously reported Egfl7<sup>/−</sup> mice. These results clearly indicate that miR-126 is required for angiogenesis and maintenance of vascular integrity in mice, while the in vivo functions of Egfl7 might be masked by its paralog Egfl8. This controversy highlighted the importance of minimally disruptive gene targeting strategies, because of the existence of non-coding RNAs including miRNAs in the introns of protein coding genes. The knockout phenotypes of genes with intronic miRNAs should be carefully interpreted or revisited.

Neoangiogenesis is essential for vascular regeneration response to injury, such as myocardial infarction (MI). miR-126<sup>−/−</sup> mice also showed reduced survival and defective cardiac neovascularization following MI, suggesting a critical function of miR-126 in neoangiogenesis [44]. The proangiogenic action of miR-126 is mediated by promoting MAP kinase and PI3K signaling.
in response to VEGF and FGF, through targeting negative regulators of the pathways, including the Sprouty-related protein Spred-1 and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85-β) (Fig. 1F) [44-46]. These findings illustrate that a single miRNA can regulate vascular integrity, angiogenesis and neovascularization by targeting multiple components in angiogenic pathways, suggesting a novel strategy in targeting miRNAs in vascular disorders.

Besides its function in angiogenesis, miR-126 also targets vascular cell adhesion protein 1 (VCAM-1), therefore regulating the adhesion of leukocytes to endothelium [28]. This suggests a role for miR-126 in vascular inflammation. miR-126 was also reported to be downregulated in many cancer lines and to inhibit tumorigenesis and cancer cell invasion [51-55]. Since miR-126 is expressed specifically in EC and hematopoietic cell lineages under normal conditions, whether miR-126 expression in those cancer cells reflects the lineage of the cancers is not known. The overexpression phenotypes of miR-126 should be carefully teased out from the endogenous function of miR-126 in ECs. For example, miR-126 targets VEGF, PIK3R2 and adapter protein CRK in cancer cells. However, unlike paracrine VEGF signaling, autocrine VEGF signaling in ECs is required for vascular homeostasis but not angiogenesis [56]. PIK3R2 can enhance PI3K-AKT signaling in cancer cells but repress PI3K-AKT signaling in ECs [46, 50, 54]. Taken together, these results indicate miR-126 is a multifunctional miRNA with important roles in angiogenesis, tumor growth and invasion, and vascular inflammation.

**miR-132**

miR-132 is highly expressed in the endothelium of human tumors and hemangiomas, but undetectable in normal endothelium [57]. In ECs, the expression of miR-132 is upregulated by VEGF and FGF. miR-132 overexpression lead to increased proliferation and tube formation in a three-dimensional matrix, whereas inhibition of miR-132 had the opposite effect. Administration of anti-miR-132 to mice reduced vascularization of the postnatal retina and subcutaneous Matrigel plug *in vivo*, even though ECs are exposed to potent angiogenic factors in these systems. p120RasGAP is a relevant target gene for miR-132 angiogenic function (Fig. 1G). p120RasGAP can catalyze the conversion of the active, GTP-bound Ras to the inactive, GDP-bound form, and has been shown to be a negative regulator of angiogenesis. Support for p120RasGAP as a target mediating miR-132 angiogenic action came from the finding that anti-miR-132 failed to decrease angiogenesis in mice in which Rasa1 (encoding p120RasGAP) was
inactivated globally after birth. Consistently, these two molecules were reciprocally expressed in tumor and the quiescent normal vasculature, with p120RasGAP highly present in quiescent normal vascular tissue and miR-132 enriched in tumor vasculature. Targeting miR-132 with nanoparticles, which deliver anti-miR-132 to ECs in vivo, increased p120RasGAP expression, and lead to marked reduction in VEGF-mediated tumor angiogenesis as well as significantly decreased tumor burden in mouse models. These studies established miR-132 as angiogenic microswitch for pathological angiogenesis, offering potential new avenues for anti-angiogenic therapy for tumors.

**miR-200 family**

The miR-200 family is comprised of 5 members (miR-200a, miR-200b, miR-200c, miR-429 and miR-141) that are located in two different loci in vertebrate genomes. Members of this family play critical roles in epithelial-mesenchymal transition (EMT) [58-59]. Recent work established miR-200b as an anti-angiomiR [60-62]. miR-200b was downregulated by hypoxia and high glucose in ECs. miR-200b, but not its cluster members miR-200a and miR-429, was also significantly decreased in the retina of STZ-induced diabetic rats and diabetic retinopathy (DR) patients [60-61]. Overexpression of miR-200b by specific miRNA mimic prevented Matrigel and glucose-induced EC tube formation [60-62], while miR-200b depletion in ECs caused elevated angiogenesis in vitro [62]. VEGF and its receptors VEGFR1 and VEGFR2, as well as the transcription factor Ets-1, were shown to be miR-200b targets (Fig. 1H). Accordingly, miR-200b overexpression inhibited VEGF-induced ERK phosphorylation. Among these target genes, overexpression of Ets1 rescued miR-200b mimic-mediated angiogenic effect and relieved miR-200b repression of VEGFR2 and MMP-1, suggesting Ets-1 might be a major miR-200b target mediating its angiogenic function. Excitingly, intravitreal injection of miR-200b mimic prevented diabetes-induced VEGF expression and subsequent increase in vascular permeability in the retina, suggesting that miR-200b might be a new mechanism for DR therapeutics.

miR-200c, another member of miR-200 family, was recently shown to be upregulated by oxidative stress, a risk factor for numerous vascular diseases, including diabetic vasculopathy [63]. Overexpression of miR-200c induced EC growth arrest, apoptosis and senescence; these phenomena were also induced by hydrogen peroxide and were partially rescued by miR-200c inhibition. Based on the roles of miR-200 family in EMT and angiogenesis, restoration of miR-200 expression may also represent an attractive approach
to prevent vascular diseases, including diabetic retinopathy, tumor angiogenesis and metastasis.

miR-210

miR-210, is the founding member of the “hypoxamirs”, a set of miRNAs upregulated by hypoxia [64]. miR-210 has pleiotropic functions and is a crucial regulator of angiogenesis and EC survival response to hypoxia. Overexpression of miR-210 in ECs stimulated angiogenesis and VEGF-induced cell migration. Conversely, blockade of miR-210 inhibited capillary tube formation in response to hypoxia. Ephrin-A3 is a relevant target of miR-210 in regulating hypoxia response (Fig. 1I) [27, 65]. Overexpression of a mutated Ephrin-A3 allele that is not targeted by miR-210 prevented miR-210-mediated stimulation of both tubulogenesis and chemotaxis. Ephrin ligands and their Eph receptors have been shown to play a crucial role in cardiovascular development and remodeling. A recent study demonstrated that overexpression of miR-210 in cardiomyocytes upregulated several angiogenic factors and prevented apoptosis [66]. miR-210 also promoted mesenchymal stem cell survival in response to ischemia preconditioning by blocking caspase-8-associated protein-2 (Casp8ap2) [67]. Intramyocardial injection of minicircle vector containing miR-210 precursor improved cardiac function in a murine model of myocardial infarction [66]. Ptp1b, a prototype for the protein tyrosine phosphatase family, was identified as a miR-210 target protein in addition to Ephrin-A3 (Fig. 1J). Ptp1b has been shown to enhance apoptosis in cardiomyocytes but repress VEGF signaling in ECs. The dual role of miR-210 in improving angiogenesis and inhibiting apoptosis has great therapeutic implications for treatment of ischemic heart disease and other vascular diseases.

miR-217 and miR-34a

Aging (or senescence) of the ECs or endothelial progenitor cells (EPCs) is associated with EC dysfunction and impaired angiogenic function, and has been proposed to be involved in age-related vascular disorders such as atherosclerosis [68]. A group of miRNAs, including miR-217, miR-34, miR-216, 181b, and miR-31b, have been shown to be upregulated in aging ECs [69]. Among these miRNAs, miR-217 and miR-34a were shown to modulate EC senescence and angiogenesis in vitro [69-70]. Overexpression of miR-217 and miR34a induced premature senescence-like phenotype and impaired angiogenesis by repressing Silent information regulator 1 (SirT1) in ECs and EPCs, respectively (Fig. 1J). SirT1 exerts protective effects against
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EC dysfunction by preventing stress-induced senescence, and plays a key role in angiogenesis through deacetylation of the forkhead transcription factor FoxO1 [71-72]. Overexpression of miR-217 or miR-34a increased FoxO1 acetylation through targeting SirT1, likely accounting for miR-217 and miR-34a anti-angiogenic phenotypes. Conversely, inhibition of miR-217 in old ECs ultimately reduced senescence and enhanced angiogenesis via an increase in SirT1 [69]. Moreover, miR-217 is expressed and negatively correlated with SirT1 expression level in human atherosclerotic lesions in vivo. Therefore, miR-217 might be implicated in the pathogenesis of atherosclerosis.

miR-218

The miR-218 family has two evolutionarily conserved miRNAs miR-218-1 and miR-218-2, which are embedded in intron 14 of the Slit2 and Slit3 genes, respectively, in the mouse genome [73]. Slit ligands and their Roundabout (Robo) receptors are critical regulators of axon and vascular guidance. In ECs, Slit/Robo signaling controls vessel sprouting and contributes to the stability of vascular network. The Robo receptors have been shown to inhibit or promote angiogenesis depending on the cellular context. miR-218 was shown to regulate EC migration and angiogenesis by targeting multiple components of the Slit-Robo pathway, including Robo1, Robo2, Rho GTPase activating protein 2 (SRGAP2), and heparan sulfate biosynthetic molecule GLCE (Fig. 1K) [73-74]. Overexpression of miR-218 reduced EC migration in a scratch wound assay, whereas knockdown of miR-218 had the opposite effect. These findings are in line with the report that miR-218 inhibits tumor cell migration and metastasis via repression of Robo1 [75]. Knockdown of miR-218 in the retina during the neonatal period caused hemorrhage in the retina and resulted in reduced complexity of the retinal vascular plexus [73]. Whether this phenotype is partially attributable to the influence of miR-218 on axonal pathfinding is not clear. In zebrafish, knockdown of miR-218 by morpholino reduced endocardial and myocardial cell migration, and defective heart field fusion [74]. These studies indicate that miR-218 impinges on the Slit-Robo pathway to regulate developmental processes and provide a new paradigm for miRNA-based receptor/ligand regulation.

miR-221/222

miR-221 and miR-222 share the same seed and are located in close proximity in both the mouse and human genomes. miR-221/222 was identified as anti-angiogenic miRNA based on an in vitro study that
overexpression of miR-221/222 in ECs impaired stem cell factor (SCF) induced angiogenesis and scratch wound healing [29]. C-Kit, the receptor for SCF, is a target for both miR-221 and miR-222 (Fig. 1L). Recent studies linked miR-222 to inflammation-induced angiogenesis. In ECs, miR-221 and miR-222 were downregulated by inflammatory cytokine, such as IL-3 and basic fibroblast growth factor (bFGF) [76]. Overexpression of miR-222, but not miR-221, blocked EC proliferation and migration in response to IL-3 and bFGF. Moreover, when injected into mice, Matrigel plugs containing miR-222 expressing ECs showed almost no vessel formation in response to IL-3 or bFGF. This phenotype was rescued by overexpression of miR-222 target protein signal transducer and activator of transcription (STAT) 5A (Fig. 1L). When activated by IL-3 or bFGF, STAT5A can migrate to the nucleus and regulate gene expression involved in different cell functions, including cell proliferation and migration. Interestingly, although STAT5A is a predicted target for both miR-221 and miR-222, overexpression of miR-221 didn’t cause STAT5A protein downregulation.

During atherogenesis, atherosclerotic lesion develops from early lesion to fibromuscolar and advanced lesion. Inflammation controls intrapaque neovascularization and lesion progression. miR-222 expression was negatively correlated with lesion progression, while its target protein STAT5A and vessel count were positively correlated with lesion progression, suggesting a role for miR-222 in vascular remodeling and lesion progression in atherosclerosis. VSMC proliferation and migration also play an important role in atherogenesis. miR-221, activated by platelet-derived growth factor (PDGF), has been shown to promote VSMCs switching from contractile phenotype to proliferative and migrative phenotype through its target proteins c-Kit and tumor suppressor p27Kip1 [77]. The direct involvement of miR-221 in atherosclerosis is not yet known.

EPCs play an important role in vascular repair and maintenance of vascular homeostasis through re-endothelialization and neovascularization. EPCs from patients with coronary artery disease (CAD) showed decreased pro-angiogenic miR-126, but elevated anti-angiogenic miR-221/222 and miR-92a levels [78-79]. Levels of miR-221/222 were negatively related to the number of EPCs in CAD patients. Moreover, lipid lowering therapy by atorvastatin increased EPC number and decreased miR-221/222 levels in patients with CAD [79]. These studies suggest that miR-221/222 level may at least be a good marker for CAD. It is imaginable that miR-221/222 might downregulate the differentiation and mobilization of EPC via c-Kit pathway in CAD patients. A causal role of miR-221/222 in CAD and EPC mobilization is yet to be established. It would be very interesting to dissect out the in vivo function of miR-221 and miR-222 in ECs and VSMCs, and their roles in atherosclerosis and CAD.
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miR-296

miR-296 was identified in an effort to identify miRNAs critical for tumor growth [13]. miR-296 was significantly upregulated in human ECs when exposed to glioma cells, conditional medium from tumor cells, or angiogenic factors. In vitro Matrigel and scratch wound assays suggested miR-296 to be a pro-angiomiR. Intravenous injection of miR-296 antagomiRs inhibited glioma angiogenesis in vivo. Hepatocyte growth factor-regulated tyrosine kinase substrate (HGS), is a miR-296 target mediating its angiogenic function (Fig. 1M). HGS is involved in the sorting of growth factor receptors VEGFR2 and PDGFRβ for degradation. miR-296 was upregulated in tumor ECs from human gliomas, consistent with the lower HGS expression and upregulation of VEGFR2 and PDGFRβ in glioma blood vessels. These results indicate a role for miR-296 in promoting tumor angiogenesis.

miR-378

miR-378 is embedded within PPARGC1b, which encodes PGC-1β, a transcriptional regulator of oxidative energy metabolism. miR-378 is also enriched in CD34+ hematopoietic progenitor cells, but dramatically downregulated in granulocytes, mononuclear cells, platelets, and reticulocytes [80]. When overexpressed in cancer cell lines, miR-378 increased cell survival and reduced cell death [81]. Nude mice injected with miR-378-transfected U87 cancer cells form much larger tumor than GFP-transfected cells. Moreover, the tumors from miR-378 transfected cells contained larger blood vessels. This is consistent with a report that miR-378 promotes VEGF expression by competing with miR-125a for the same seed region in the VEGF-3' UTR [16]. Suppressor of fused (Sufu) and Fus-1 are two tumor suppressors which serve as the targets for miR-378 repression (Fig. 1N). Sufu is known to be a negative regulator of Shh signaling. Shh promotes large-diameter vessel formation by inducing expression of angiogenic cytokines, including VEGF and angiopoietins-1 (Ang-1) and -2 (Ang-2). Therefore, miRNA-378 promotes cell survival by targeting Sufu and Fus-1, and promotes tumor angiogenesis by indirect upregulation of angiogenic factors.

miR-503

miR-503 is upregulated in ECs cultured in conditions mimicking diabetes mellitus (high D-glucose) and ischemia-associated starvation (low growth factors) [82]. miR-503 expression is also increased in ischemic limb muscles of streptozotocin-diabetic mice, ECs enriched from these muscles and the plasma of diabetic individuals. Overexpression of miR-503 inhibited EC
proliferation, migration, and network formation on Matrigel in vitro. Conversely, blocking miR-503 activity by either adenovirus-mediated transfer of a miR-503 decoy or by anti-miR-503 improved the functional capacities of ECs cultured under high D-glucose/low growth factors and corrected diabetes mellitus–induced impairment of postischemic angiogenesis and blood flow recovery in vivo. Cell cycle-regulating proteins cyclin E and cdc25 were identified as direct miR-503 targets and showed inverse expression pattern with miR-503 in vitro and in vivo (Fig. 1O). Taken together, miR-503 is an anti-angiomiR which may have important implications in the vascular complications of diabetes.

miR-519c

miR-519 was identified as a tumor suppressor that functions by reducing levels of the RNA-binding protein HuR [83-85]. miR-519c, but not miR-519a, was shown to be a hypoxia-independent regulator of HIF-1α, one of the key regulators of angiogenesis (Fig. 1P) [86]. Hepatocyte growth factor (HGF) can downregulate miR-519c, which in turn induce HIF-1α expression. Overexpression of miR-519c in ECs decreased HIF-1α protein level and reduced Matrigel tube formation, which was rescued by overexpression of HIF-1α mRNA lacking 3’UTR. The opposite was observed when miR-519 was knocked down in ECs. Mice injected with miR-519c-overexpressing cells showed dramatically reduced HIF-1α expression levels, followed by suppressed tumor angiogenesis, growth, and metastasis, indicating that miR-519c is a suppressor of tumor angiogenesis.

Other miRNAs

A list of miRNAs has been identified as angiomiRs by in vitro evidence (Fig. 1Q). miR-130a, a serum-inducible miRNA, promoted angiogenesis by repressing the antiangiogenic activity of its target homeobox proteins GAX and HOXA5 [87]. miR-155 overexpression blocked EC migration in response to angiotensin II by targeting angiotensin II type 1 receptor [88-89]. Overexpression of miR-181b and miR-9 repressed EC migration and tube formation by targeting Neuropilin-1 (NRP1), a coreceptor regulating VEGF and semaphorin signaling. miR-214 was identified as an anti-angiogenic miRNA by potentially targeting endothelial nitride synthase (eNOS) [90]. Inhibition of miR-320 improved angiogenesis in diabetic ECs in vitro by targeting IGF-1 protein [91]. There is much left to be investigated regarding their functions in vivo.
Emerging roles of microRNAs in angiogenesis and vascular disease

**MicroRNAs in vascular disease**

Vascular complications, such as pathological angiogenesis and VSMC phenotypic changes, are closely involved in numerous diseases, including but not limited to cancer, atherosclerosis, CAD, diabetes, and AMD, which are the leading causes of morbidity and mortality in the Western world. Targeting the aberrant vascular remodeling in these diseases has been at the forefront of therapeutic research. Recent studies have revealed important roles for miRNAs in vascular diseases. miRNAs have evolved as promising biomarkers and therapeutic targets for various vascular diseases. Here we will summarize the role of miRNAs in the vascular complications of different diseases (Table 1).

**Atherosclerosis and CAD**

Atherosclerosis is the leading cause of morbidity and mortality in the developed countries. Atherosclerosis is a disease in which plaque builds up inside arteries, which can limit the flow of oxygen-rich blood to organs and cause heart attack, stroke, or even death. If plaque builds up in the coronary arteries, CAD or coronary heart disease may result. Atherogenesis begins with endothelial dysfunction and infiltration of leukocytes into the endothelium, followed by the formation of fatty streaks, which progress into intermediate and advanced lesions and vulnerable plaques [108]. Chronic inflammation, VSMC phenotypic switch and neovascularization are the important processes involved in atherosclerotic lesion development and plaque rupture.

Recent studies have suggested a role for miRNAs in atherosclerosis [109]. Neointima formation is characteristic of diseases like atherosclerosis, postangioplasty restenosis, and graft vasculopathy. A miRNA expression signature for neointima formation was revealed using a rat carotid artery (CA) balloon injury model [95]. Many miRNAs were shown to be dysregulated in the vascular wall in this model. Among them, miR-21, miR-214 and miR-221/222 were upregulated, while miRs-143 and -145 were down-regulated after balloon injury [95, 100]. miR-143/145 was also downregulated in aortic constriction (TAC) and ApoE<sup>−/−</sup> atherosclerosis mouse models, as well as human patients with aortic aneurism [101]. miR-217 was recently shown to be expressed in human atherosclerotic lesions and negatively correlated with the expression its target SirT1 [69]. In adult swine, miR-10a was shown to be expressed less in the athero-susceptible regions of the inner aortic arch and aorto-renal branches than elsewhere and was negatively correlated with its target protein HOXA1 [92]. In contrast to the
Table 1. microRNAs implicated in vascular disease.

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Regulation in Disease Models</th>
<th>Function or Implications</th>
<th>Targets</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-10a</td>
<td>In atherosclerosis-prone aortic arch</td>
<td>Inhibit EC inflammation</td>
<td>HoxA1, TAK1, BTRC</td>
<td>[92]</td>
</tr>
<tr>
<td>miR-92a</td>
<td>After CA or FA ligation, or in EPCs in CAD patients</td>
<td>Repress vessel growth after ischemic damage</td>
<td>Integrin α5</td>
<td>[31, 78, 93]</td>
</tr>
<tr>
<td>miR-93</td>
<td>In the plasma of CAD patients</td>
<td>Diabetic nephropathy</td>
<td>VEGF</td>
<td>[94]</td>
</tr>
<tr>
<td>miR-21</td>
<td>In the neointimal lesion in CA</td>
<td>Required for neointima formation</td>
<td>PTEN, Bel-2</td>
<td>[95]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Repress laser-induced CNV</td>
<td>RhoB</td>
<td>[37]</td>
</tr>
<tr>
<td>miR-23/27</td>
<td>After laser injury in the eye</td>
<td>Required for laser-induced CNV</td>
<td>Sprouty2, Sema6A</td>
<td>[40]</td>
</tr>
<tr>
<td>miR-100</td>
<td>After FA ligation</td>
<td>Required for repressing angiogenesis</td>
<td>mTOR</td>
<td>[43]</td>
</tr>
<tr>
<td>miR-126</td>
<td>In EPCs and plasma of CAD patients</td>
<td>Required for neovascularization in mice</td>
<td>Spry1, Pl3KR2</td>
<td>[44, 78, 93]</td>
</tr>
<tr>
<td></td>
<td>In the plasma of type II diabetic patients</td>
<td>Repress atherosclerotic lesion in mice</td>
<td>RGS-16</td>
<td>[96]</td>
</tr>
<tr>
<td>miR-143/145</td>
<td>In neointima, atherosclerotic lesion, and patients with aortic aneurism</td>
<td>Regulate blood pressure in mice.</td>
<td>ACE</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>In obesity associated diabetic mice</td>
<td>Promote or inhibit neointimal growth</td>
<td>KLF4, KLF5, myocardin</td>
<td>[98-102]</td>
</tr>
<tr>
<td>miR-155</td>
<td>In hypertensive patients with ATIR CC genotype, and in plasma of CAD patients</td>
<td>Hypertension</td>
<td>AT1R</td>
<td>[93, 104-106]</td>
</tr>
<tr>
<td>miR-200b</td>
<td>In the retina of STZ-induced diabetic rats and diabetic retinopathy patients</td>
<td>Diabetic retinopathy</td>
<td>VEGF, VEGFR1, VEGFR2</td>
<td>[60-61]</td>
</tr>
<tr>
<td>miR-217</td>
<td>Expressed in the atherosclerotic lesion</td>
<td>Atherosclerosis</td>
<td>siT1</td>
<td>[69]</td>
</tr>
<tr>
<td>miR-222</td>
<td>During atherosclerotic lesion development.</td>
<td>Atherosclerosis</td>
<td>STAT5A,</td>
<td>[76, 107]</td>
</tr>
<tr>
<td>miR-221/222</td>
<td>In the neointimal lesion in CA and CAD patients</td>
<td>Required for neointimal lesion formation</td>
<td>P27(Kip1), P57(Kip1)</td>
<td>[78]</td>
</tr>
<tr>
<td>miR-322 or 424</td>
<td>After CA and FA ligation</td>
<td>Promote neangiogenesis</td>
<td>CUL2</td>
<td>[17]</td>
</tr>
<tr>
<td>miR-503</td>
<td>In the plasma of type II diabetic individuals, and muscles or ECs from diabetic mouse model</td>
<td>Biomarker for diabetes, implication in diabetic vasculopathy</td>
<td>Cyclin E, Cdk25</td>
<td>[82]</td>
</tr>
</tbody>
</table>

Abbreviations: CA--carotid artery; FA--femoral artery; CAD--coronary artery disease; CNV--choroidal neovascularization.

In the rat balloon injury model, miR-222 expression in ECs was decreased during atherosclerotic lesion progression in humans and was inversely correlated with upregulation of its target protein STAT5A [76]. This might suggest different regulation mechanisms for miR-222 in VSMCs and ECs in the lesions. Alternatively, this may suggest the intrinsic limitation of using acute balloon injury model to model human atherosclerotic lesion.
Emerging roles of microRNAs in angiogenesis and vascular disease

Occlusion of atherosclerotic coronary artery by thrombosis may result in acute myocardial ischemia, which causes angina or myocardial infarction (MI). A miRNA expression pattern was also identified after acute myocardial infarction or myocardial ischemia–reperfusion in experimental mouse models [110-111]. Interestingly, miR-21 and miR-214 are upregulated in both models. miR-92a and miR-322 was shown to be upregulated in coronary artery and femoral artery (FA) ligation models, while miR-100 was downregulated in FA ligation model [17, 31, 43]. EPCs play an important role in vascular repair and maintenance of vascular homeostasis. Anti-angiogenic miRNAs miR-92a and miR-221/222 levels were elevated, while pro-angiogenic miRNA miR-126 was dramatically reduced in EPCs from human CAD patients [78]. It would be interesting to see whether these changes contribute to the angiogenic defects in CAD.

Recently, miRNAs have been detected in human plasma or serum as stable biomarkers for cancer and other diseases [112-113]. Plasma miRNAs exist in microvesicles, which can originate from tumor cells, platelets, monocytes or ECs [96, 114-115]. Recent circulating miRNA profiling studies have shown that several vascular miRNAs, including miR-126, miR-17, miR-92, miR-145, and the inflammation-associated miRNA miR-155 are significantly downregulated in the plasma of CAD patients [93]. In isolated peripheral blood mononuclear cells from CAD patients, miR-135a levels were increased, while miR-147 levels were decreased [116]. Moreover, a cluster of three microRNAs (including miR-134, miR-198, and miR-370) could discriminate unstable angina pectoris patients from stable patients, suggesting microRNA signatures might be utilized to identify patients with atherosclerotic CAD in general and those at risk for acute coronary syndromes. As to the function of individual circulating miRNAs in vascular disease, miR-126, enriched in EC-derived apoptotic bodies, was recently shown to protect against atherosclerotic plaque formation in mice by promoting the recruitment of Sca-1+ progenitor cells from the bone marrow to the lesion [96]. This effect is mediated by inducing the production of chemokine CXCL12 via repression of regulator of G protein signaling 16 (RGS-16), an inhibitor of G protein–coupled receptor (GPCR) signaling. This study highlights the importance of miR-126 in preventing atherosclerosis through recruiting progenitor cells for tissue repair or homeostasis.

Inflammation, mediated by leukocyte infiltration into endothelium and activation of macrophages, is observed at all stages of atherosclerosis. Leukocyte infiltration is a critical early step in atherogenesis. Knockdown of miR-126 was shown to upregulate vascular cell adhesion molecule (VCAM)-1, which in turn enhanced leukocyte adherence to the endothelium [28]. miR-10a was considered to be anti-inflammatory by repressing IκB/NFκB-
mediated inflammation in ECs [92]. Two key regulators of IκBα degradation-
mitogen-activated kinase kinase kinase 7 (MAP3K7; TAK1) and
β-transducin repeat-containing gene (βTRC) are the relevant miR-10a targets
in the process. Also, TNF-induced miRNAs miR-31 and miR-17-3p are
required and sufficient to repress neutrophil adhesion to cultured ECs by
targeting E-selectin and ICAM-1, respectively [117]. With respect
to macrophages, miR-125a-5p has been suggested to be anti-atherogenic by
repressing the secretion of inflammatory cytokines (TGF-β, TNF-α, IL-2, and
IL-6) and macrophage scavenger receptors (LOX-1 and CD68) in macrophage
[118]. miR-147 can also prevent excessive inflammatory response in
macrophages stimulated by Toll-like receptor activation [119]. Clearly, these
miRNAs might have therapeutic implications in atherosclerosis.

VSMC phenotypic switch from a contractile state to a proliferative state
is a significant contributor to vascular hyperplasia and neointima formation in
atherosclerosis. Knockdown of miR-21 or miR-221/222 and overexpression
of miR-143/145 each inhibited neointima formation in a balloon injury model
[95, 99, 101, 107]. miR-143/145 knockout mice developed neointima lesion
in FAs at 18 months of age, but were surprisingly resistant to neointima
formation in response to balloon injury, suggesting a buffering mechanism
for miR-143/145 in neointima formation after injury. miR-21 promotes
proliferation but represses apoptosis of VSMCs, an effect possibly mediated
by its targets PTEN and BCL2 [95]. miR-221/222 enhances VSMC
proliferation via their target proteins p27(Kip1) and p57(Kip2) [107].
miR-143/145 controls VSMC plasticity by promoting differentiation and
repressing proliferation of VSMCs [98, 100-102]. Multiple genes involved in
the regulation of serum response factor (SRF) activity and actin dynamics,
including KLF4, KLF5, are validated miR-143/145 targets for regulating
VSMC plasticity. Therefore, the recent identification of miR-143/145 as a
major regulator of VSMC phenotypic switch may open a new avenue for
influencing vascular repair and attenuating arteriosclerotic pathogenesis.

Angiogenesis is closely related to plaque destabilization and rupture in
atherosclerosis. On the other hand, neoangiogenesis is essential for the repair
and recovery following the ischemia caused by the occlusion of
atherosclerotic arteries. As detailed in this chapter, a growing list of miRNAs
has been shown to regulate angiogenesis, and was implicated in
atherosclerosis and ischemic vascular diseases. Our earlier work using
miR-126<sup>−/−</sup> mice indicated that miR-126 is critical for survival and
neovascularization following myocardial infarction (MI) [44]. While seventy
percent of the wild-type mice survived at three weeks after MI,
approximately eighty percent of the miR-126<sup>−/−</sup> mice died due to heart failure
and rupture of the ventricle, which is consistent with the paucity of neovessels
in the infarct zone in the miR-126−/− hearts. miR-92a has been shown to control neoangiogenesis in mice [31]. miR-92a was induced by ischemic injury, and blockade of miR-92a by antagomiR-92a increased recovery of the blood flow in hindlimb ischemia model and improved heart function in MI model. Another miRNA, miR-100, functions as an anti-angiomiR but was downregulated in the ischemic limb [43]. It would be interesting to test whether knockdown of both miR-92a and miR-100 has a synergistic effect on improving neoangiogenesis after ischemic injury.

**Hypertensive vascular disease**

Hypertension is a potentially lethal vascular disease that can be causative or secondary to other vascular diseases. The renin-angiotensin system is the body's most important blood pressure regulation system. Angiotension II (angII), is a vital component of the system, regulating vasoconstriction and renal sodium reabsorption and also functioning as a cytokine for vascular and cardiac cells predominantly through the type 1 receptor (AT1R). Recent studies have linked miRNAs to hypertension via regulation of this pathway. miR-155 has been shown to target AT1R 3’UTR for repression through the site of the polymorphism [105]. The repression of AT1R by miR-155 was attenuated when a silent polymorphism in the 3’UTR was present. miR-155 expression was decreased in the aorta of spontaneously hypertensive rats and in hypertensive patients with the silent polymorphism in AT1R [106, 120]. Furthermore, miR-155 expression in the rat aorta or patient peripheral blood mononuclear cells was negatively correlated with blood pressure. The regulation of AT1R by miR-155 has functional significance in multiple cell types. By targeting At1R and Ets1, miR-155 decreased EC migration and adhesive properties in response to AngII [88]. In vascular adventitial fibroblasts, miR-155 attenuated AngII-induced phenotypic differentiation [121]. Downregulation of miR-155 in human VSMCs induced endogenous AT1R expression and angII-induced ERK1/2 activation [105]. These results suggest that miR-155 might be involved in hypertension disease progression.

Separately, proliferation and hypercontraction of VSMCs are closely associated with hypertension. VSMC-enriched miR-143/145 functions to promote differentiation and repress proliferation of VSMCs. Interestingly, miR-143/145−/− mice displayed reduced blood pressure at baseline and in response to AngII due to the compromised VSMC contractile function [98, 101-102]. Angiotensin-converting enzyme (ACE) was identified by an unbiased proteomic approach to be the target of miR-143/145 regulating the contractility of VSMCs [98].
Diabetic vasculopathy

Diabetes mellitus is the most common metabolic disorder, estimated to affect more than twenty-five million Americans. Vascular lesions that develop over time are closely associated with the morbidity and mortality associated with diabetes [122]. In the developed countries, diabetes is the leading cause of blindness, renal failure, lower limb amputation, and is a major risk factor for cardiovascular disease and stroke. Growing evidence indicates that miRNAs are involved in the pathogenesis of diabetes. Changes of miRNA expression have been identified in tissues from different diabetic animal models [123]. Here we summarize the miRNAs that are potentially involved in the vascular changes in diabetes.

Hyperglycemia is a hallmark of both type 1 and type 2 diabetes. Persistent hyperglycemia causes endothelial dysfunction and impaired angiogenesis, accounting for the vascular complications of diabetes. High glucose has been shown to upregulate miR-503 and miR-221, while downregulating miR-93 and miR-200b in ECs [61, 82, 94, 124]. miR-503 expression was further upregulated in ECs cultured under high glucose and low growth factor condition in vitro, and ECs from ischemic limb muscles from streptozotocin-diabetic mice and the plasma of diabetic individuals [82]. Downregulation of miR-93 and miR-200b was observed in the retinas and kidneys isolated from the diabetic animal models respectively, while miR-200b downregulation was also found in the retinas from patients with diabetes [61, 94]. Also, miR-320 was upregulated in microvascular ECs isolated from diabetic rats [125]. Strikingly, a signature pattern of plasma miRNA was identified in diabetes [97] in which 13 miRNAs were found to be differentially expressed in diabetic patients. Among them, the expression of five miRNAs (miR-15a, miR-28-3p, miR-126, miR-223, and miR-320) was altered before the manifestation of the disease. The determination of the level of these 5 miRNAs was sufficient to identify seventy percent of the type 2 diabetic patients. These results suggest that blood miRNA signature could help distinguish individuals with diabetes from healthy controls.

Functionally, miR-93 and miR-200b target VEGF-A both in vitro and in vivo, and may have implications in diabetic nephropathy and retinopathy [94]. miR-221 and miR-320 are at least partly responsible for the inhibitory effect of high glucose on EC migration and angiogenesis [61, 91]. miR-503 functions to inhibit EC proliferation, migration, and angiogenesis [82]. Blocking miR-503 activity corrected diabetes–induced impairment of post-ischemic angiogenesis and blood flow recovery in mice. These miRNAs may have important implications in diabetes-associated vascular complications and may represent novel therapeutic targets.
Neovascular AMD

Age-related macular degeneration (AMD) is a degenerative disease of the retina and the leading cause of irreversible vision loss in the elderly [126], afflicting about two million Americans. Choroidal neovascularization (CNV) in neovascular (wet) AMD, which involves abnormal vessel growth in the back of the eye, accounts for ninety percent of the severe vision loss in AMD. The role of miRNAs in neovascular AMD is largely unknown, although recent work has suggested that miRNA pathways are not heavily involved in geographic atrophy in dry AMD [10]. A growing list of miRNAs has been shown to regulate angiogenesis and neovascularization; therefore, they may have a role in choroidal neovascularization in wet AMD. A group of miRNAs have been shown to be substantially decreased in a laser-induced CNV model [127]. Overexpression of miR-31, miR-150 or miR-21 represses laser-induced CNV, supporting a functional role for miRNAs in neovascularization in the eye [37, 127]. In addition, we recently found that members of miR-23~27~24 clusters were upregulated in the retina/choroid in a laser-induced CNV mouse model [40]. Importantly, silencing of miR-23 and miR-27 in the family repressed laser-induced CNV by about half, suggesting these two miRNAs might have therapeutic potential in neovascular AMD. miR-23 and miR-27 regulate MAPK and VEGFR angiogenic pathways by targeting Sprouty2 and Sema6A. Whether miR-23 and miR-27 regulate both angiogenic and inflammatory pathways in CNV is not yet known [128]. Overall, the study of miRNAs in neovascular AMD is still at its early stage, and a more systematic study of miRNA function in the disease processes is warranted.

Concluding remarks

The identification of miRNAs as key regulators of angiogenesis and vascular disease has opened a new avenue for the therapeutics of vascular diseases, such as atherosclerosis, hypertensive vascular disease, diabetic vasculopathy and neovascular AMD. Signature expression profiles of miRNAs have been observed in many vascular disease models. Many miRNAs have functional implications in the pathogenesis of the diseases. Targeting angiomiRs and other related miRNAs by anti-miRs or miRNA mimics may represent a promising therapeutic approach for these diseases. miRNA therapeutics have the advantage of targeting and normalizing the expression of multiple disease genes, potentially avoiding the toxicity or drug resistance caused by without turning on/off a single target. Therapies based on anti-miRs or miRNA mimics have demonstrated efficacy in non-human primates [129-130]. Since miRNAs may have tissue or cellular context-
dependent function in angiogenesis, future miRNA-based therapeutics should target miRNAs in a tissue or cell-type specific manner.

References


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