ATVB in Focus
MicroRNAs: From Basic Mechanisms to Clinical Application in Cardiovascular Medicine

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MicroRNA Control of Vascular Endothelial Growth Factor Signaling Output During Vascular Development

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Abstract—The regulated response of endothelial cells to signals in their environment is not only critical for the de novo formation of primordial vascular networks during early development (ie, vasculogenesis), but is also required for the subsequent growth and remodeling of new blood vessels from preexisting ones (ie, angiogenesis). Vascular endothelial growth factors (Vegfs) and their endothelial cell-specific receptors play a crucial role in nearly all aspects of blood vessel growth. How the outputs from these pathways affect and coordinate endothelial behavior is an area of intense research. Recently, numerous studies have highlighted roles for microRNAs in modulating Vegf signaling output in several different contexts. In this review, we will provide an overview of how small RNAs regulate multiple aspects of the Vegf signaling pathway. In particular, we highlight areas where identification of microRNAs and their targets has provided new insight into the role of downstream effectors in modulating Vegf output during development. As Vegf plays a broad role in multiple aspects of endothelial biology and has become a target for therapeutic manipulation of pathological blood vessel growth, microRNAs that affect Vegf signaling output will undoubtedly be major targets of clinical value. **(*Arterioscler Thromb Vasc Biol*. 2013;33:193-200)**

Key Words: mircoRNA ■ angiogenesis ■ Vegf ■ endothelial cell ■ post-transcriptional

Formation of the vertebrate circulatory system requires coordination of diverse cellular behaviors during embryonic development.[1] During vasculogenesis, endothelial cell (EC) progenitors must balance proliferation, differentiation into distinct lineages (eg, artery and vein), and migration to the appropriate anatomical location to establish a primitive vascular network de novo.[2] Subsequent angiogenesis entails the sprouting and growth of new blood vessels from this preexisting network, which also requires coordination of proliferation, migration, and dynamic regulation of tip and stalk cell identities within growing angiogenic sprouts.[3] During development, these growth processes must be carefully coordinated with lumen formation, and the integration of hemodynamic forces provided by the initial onset of circulatory flow.[4] Not surprisingly, perturbation of any of these steps has detrimental effects on circulatory function in the embryo. Likewise, many of these same processes are used in the context of pathological vascular growth.[5] Thus, a better understanding of the signaling pathways that govern blood vessel growth and homeostasis will undoubtedly have clinical benefits.

Over the last 2 decades, we have learned a great deal about the signals required for vascular growth in both embryonic and adult settings. These studies revealed an important role for many of the central developmental signaling pathways, such as Notch, BMP, and Wnt, in multiple aspects of vascular development and function.[1,5–7] In addition, several EC-specific pathways have been identified. Most notable in this regard are receptor tyrosine kinases for vascular endothelial growth factor (Vegf) and angiopoietin ligands.[8] In particular, ligands of the Vegf family play an integral role in nearly every aspect of vascular development and maintenance.[9] Indeed, loss of even a single copy of *Vegfa* completely blocks vessel formation in mouse embryos leading to embryonic lethality,[10,11] whereas loss of this ligand in the endothelium of adult vasculature causes severe endothelial dysfunction,[12] and inhibition of Vegfa signaling potently inhibits pathological angiogenesis.[13] Vegf ligands can elicit a wide range of responses in ECs, including migration, proliferation, survival, differentiation, and increased permeability.[9] Given the importance of targeting this pathway in a variety of clinical settings,[8] a detailed understanding of how Vegf ligands elicit a particular response in vivo is of great importance. Over the past several years, there has been an increasing amount of evidence implicating microRNAs (miRs) in the control of Vegf signaling output at multiple levels. Indeed, miRs are now known to control the expression of Vegf ligands, receptors, and intracellular signaling components of the Vegf pathway, as well as proteins that cross-talk with Vegf receptors. Here, we will review the most recent findings regarding regulation of Vegf signaling output by miR.

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Regulation of Angiogenesis by MiRs

Originally identified in *Caenorhabditis elegans* in 1993,[14] miRs are a group of highly conserved, noncoding single-stranded RNA molecules (≈21–23 nucleotides) that function to fine-tune protein expression through various mechanisms, including the degradation of target mRNA, the inhibition of translation elongation, or the sequestration of target mRNAs away from the translation machinery.[15–17] This is accomplished by the incorporation of the miR into the RNA-Induced Silencing Complex (RISC),[18] which is then directed primarily to the 3′-untranslated region of the target mRNA via the seed sequence of the miR. Although miRs may affect the expression of hundreds of target genes,[19] a small number of target mRNAs may adequately explain the biological effect of a particular miR in a given cellular context.[20] Numerous studies have also demonstrated that miRs can target several genes within the same pathway to robustly control biological responses.[21]

MiRs have been shown to play key roles in vascular development. For example, deletion of *Dicer*, an enzyme required for miR biogenesis, results in early embryonic lethality,[22] whereas mice homozygous for a hypomorphic allele of *Dicer* survive to mid-gestation but have major defects in angiogenesis.[23] Conditional deletion of *Dicer* in the endothelium results in reduced vascular growth in postnatal models of tumor angiogenesis and ischemia.[24] Although these mice did not display angiogenic defects during development, Dicer was not ablated from all ECs in this study,[24] and it remains possible that full deletion of Dicer may well result in developmental vascular defects. In addition to these developmental studies, knockdown of Dicer in cultured ECs has been shown to result in significant defects in their responsiveness to angiogenic factors.[25]

Control of Vegf Ligand and Receptor Expression by MiRs

The Vegf family is composed of several ligands (Vegfa, b, c, d, and e, and Placental growth factor), each of which has a particular preference for 1 of 3 endothelial-expressed receptors (Vegfr-1/Flt1, Vegfr-2/Flk1/Kdr, and Vegfr-3/Flt4).[9] Regarding vascular development, Vegfa is the most functionally significant and well-characterized member of the Vegf family. Upon binding to its receptor tyrosine kinase, Vegfr-2, Vegfa can elicit distinct responses, including proliferation, cell survival, permeability, differentiation, and migration.[9] These effects are mediated through the activation of several downstream signaling effectors.[9] Homozygous deletion of *Vegfr-2*,[26] much like deletion of its ligand,[10,11] results in an absence of blood vessels and lethality in mice. Vegfr-3, which becomes almost exclusive to lymphatic ECs during late development,[27] is also expressed on vascular endothelial tip cells during embryonic and postnatal angiogenesis, and responds to Vegfc and Vegfd.[28,29] *Vegfr-3* deletion also leads to embryonic lethality, which is due to a failure of vascular remodeling,[30] and inhibition of Vegfr-3 activity can inhibit tumor angiogenesis.[28] In contrast to Vegfr-2 and Vegfr-3, Vegfr-1 largely functions as a decoy receptor to negatively regulate Vegf signaling during development.[31,32] The *Vegfr-1* gene encodes an alternative soluble isoform that lacks the intracellular domain and has a much higher affinity for Vegfa than Vegfr-2.[33] Accordingly, mice bearing a null allele of *Vegfr-1* exhibit extensive vascular overgrowth, whereas those bearing a deletion of only the tyrosine kinase domain display normal vascular development.[34] Importantly, the levels of Vegfr-2 or -3 versus Vegfr-1 appear to be both spatially and temporally regulated during angiogenesis. Thus, the proper balance and regulation of Vegf receptor levels plays an important role in orchestrating angiogenic sprouting.[31,32]

ECs are highly sensitive and responsive to Vegf gradients. These gradients are established through the alternative splicing of *Vegfa* transcripts, which results in an array of protein variants with diverse functional properties, such as differential diffusion rates, as well as variable association with the extracellular matrix, leading to distinct abilities to activate cell-signaling pathways and to induce vascular morphogenesis.[35] In humans, Vegfa isoforms contain 121, 165, 189, or 206 amino acids; all except $Vegfa_{121}$ contain a basic stretch near the carboxyl terminus with variable affinity for heparan sulfate proteoglycans (Hspg) and Neuropilin-1, coreceptors for Vegfa.[35] As a result, $Vegfa_{121}$, which is unable to bind Hspg, is freely diffusible and can influence EC proliferation, although contributing little to EC migration.[36] On the contrary, $Vegfa_{165}$ and $Vegfa_{189}$, which have strong affinities for Hspg, are tightly associated with the extracellular matrix and form a gradient to allow for the directional migration of ECs by promoting filopodia extensions.[36] One possible explanation for the vast array of cellular responses elicited by different Vegfa isoforms is the preferential activation of one downstream signaling effector over another. For example, unlike its soluble counterpart, matrix-bound Vegfa can induce prolonged tyrosine kinase activity of Vegfr-2 leading to increased phosphorylation of p38/mitogen-activated protein kinase, thereby upregulating angiogenic sprouting. This is mediated by interaction between Vegfr-2 and β1-integrins.[37]

Given the considerable dynamic control of Vegf ligand and receptor expression during vascular development, it is not surprising that miRs can control this pathway by targeting transcripts encoding these proteins (Figure). As enhanced expression of Vegfa occurs in diseases such as cancer and diabetic retinopathy, and drives pathological vascular growth,[38] alterations in miR expression may contribute to these diseases. Indeed, miR-93[39] and miR-200b[40] were recently found to be downregulated by hyperglycemic conditions. These miRs both target the *Vegfa* 3′-untranslated region, and knockdown of these miRs enhances Vegfa expression in vivo.[39,40] MiR-15a also directly represses Vegf and fibroblast growth factor 2 in ECs to control angiogenesis,[41] and miR-20b represses Vegf expression in tumor cells to affect their cell survival in hypoxic conditions.[42] MiRs are also induced downstream of Vegf signaling itself.[24] For example, Vegf induces the expression of miR-16 and miR-424, which share a common seed sequence.[43] These miRs act in a negative feedback loop to control angiogenesis through combined targeting of Vegfa, Vegfr-2, and the fibroblast growth factor receptor, Fgfr1.[43] Other miRs, such as miR-296, indirectly regulate Vegfr-2 expression.[44] Vegfr-2 turnover is regulated by hepatocyte growth factor (Hgs)–regulated tyrosine kinase substrate, which mediates sorting of ligand/receptor complexes to lysosomes for degradation.[45] MiR-296 expression is enhanced in tumor vasculature and is induced by

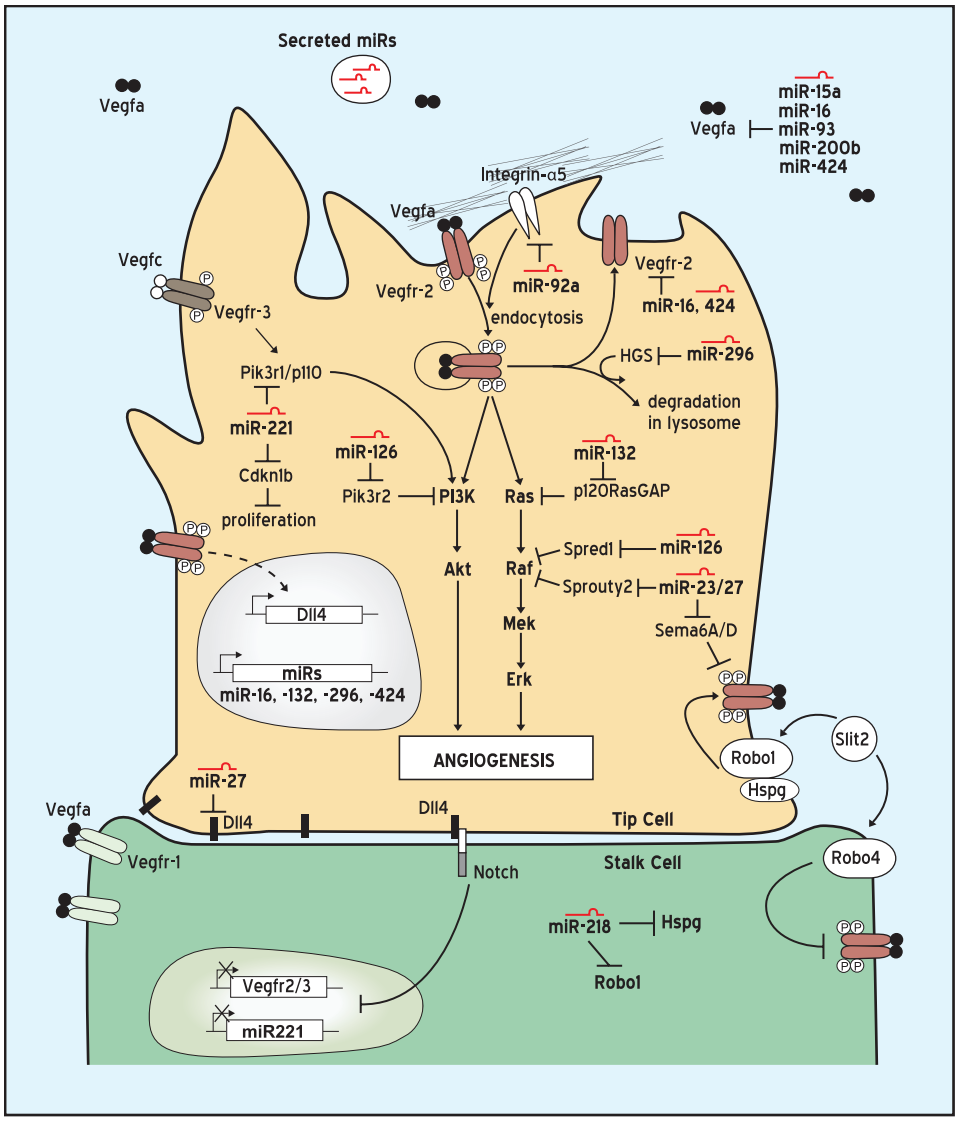

Figure. MicroRNAs (miRs) impinge on vascular endothelial growth factor (Vegf) signaling to regulate angiogenesis. Binding to their receptor tyrosine kinases (Vegfr-2 and Vegfr-3), Vegfa and Vegfc can drive angiogenesis through the activation of several downstream signaling effectors (eg, phosphoinositide-3-kinase [PI3K] and mitogen-activated protein kinase/extracellular signal-regulated kinase [ERK]). The output of these effectors is modulated by miRs. In particular, recent evidence highlights the important roles of miRs in controlling Vegf signaling by titrating the levels of Vegf ligand (miR-15a, -16, -93, -200b, and -424), Vegf receptors (miR-16 and -424), as well as positive and negative regulators of the Vegf signal transduction cascade (miR-23, -27, -126, -132, -218, and -221). The identification of these regulatory miRs has emphasized the importance of fine-tuning both the PI3K and mitogen-activated protein kinase signaling pathways downstream of Vegf signaling and has provided new insights into how different outputs can be modulated through posttranscriptional control.

Vegf signaling, and through its repression of Hgs expression in ECs, miR-296 facilitates enhanced expression of Vegfr-2 on the cell surface and potentiates Vegf signaling.[44]

Control of Intracellular Signaling Downstream of Vegf by MiRs

In addition to regulation at the level of ligand and receptor, several studies reveal that intracellular signaling effectors used downstream of Vegf are targeted by miRs (Figure). In particular, this work has underscored the importance of both phosphoinositide-3-kinase (PI3K) and mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) signaling pathways in modulating Vegf signaling outputs during vascular development.

PI3K activity is an essential downstream effector of Vegf signaling during vascular development.[46] Class I PI3Ks are heterodimers composed of a catalytic subunit that can be encoded by 4 different genes (p110α, β, δ, and γ referred to hereafter as *pik3ca*, *b*, *d*, and *g*) and a regulatory subunit, for which 5 different genes have been identified (*pik3r1–5*).[47] Notably, Pik3r1, 2, and 3 possess Src homology domain domains that mediate direct interactions with upstream receptor tyrosine kinases. *Pik3ca*, *b*, and *g*, as well as most regulatory subunits are expressed in ECs[48,49] and appear to have distinct roles in modulating Vegf output. For example, *pik3ca* is essential for Vegfa-mediated endothelial migration during developmental angiogenesis,[46] whereas *pik3cg* plays an important role in Vegfa-stimulated vascular permeability in adult vasculature.[49]

PI3Ks phosphorylate membrane-bound phosphoinositol-4,5-bisphosphate to generate phosphoinositol-1,4,5-trisphosphate, which serves as a docking and activation site for downstream signaling molecules, such as Akt1, a serine/threonine kinase. Given the importance of PI3K signaling in cell survival and growth, constitutive activation of this pathway is typically associated with cellular transformation in non-EC types. In many of these cases, activation is a result of the loss of PI3K regulatory subunits, which are thought to exist in a 1:1 ratio with the catalytic subunits and usually inhibit kinase activity in the absence of upstream activation.[50] Several recent findings suggest that these regulatory subunits are important targets of miRs to regulate Vegf signaling output in ECs. In particular, miR-126 and miR-221 have been found to target Pik3r2[51,52] and Pik3r1,[53] respectively.

MiR-126 Control of Vegfr-2 Signaling

MiR-126 was the first EC-specific miR identified and is located within intron 7 of the *Egfl7* gene.[51,54] Deletion of *miR-126* in mice (without altering the expression of the host gene) results in severe defects in blood vessel development, including delayed angiogenic sprouting, hemorrhage, and partial early embryonic lethality.[52,54] These defects are similar to phenotypes associated with loss of Vegf signaling, in both the mouse[12] and the zebrafish,[51] suggesting that miR-126 acts to modulate Vegf signaling output. Indeed, knockdown of miR-126 in ECs results in decreased phosphorylation of Akt, as well as ERK1/2, in response to Vegf treatment.[51,52,54] Furthermore, miR-126 targets transcripts encoding Pik3r2[51,52] and Spred1[51,52,54,55] (an inhibitor of mitogen-activated protein kinase signaling), providing a direct link between this miR and the Vegf signaling pathway.

Interestingly, results from zebrafish suggest that miR-126–mediated repression of Spred1 and Pik3r2 may be context dependent. Unlike mammalian genomes, which encode 1 copy of *miR-126* that does not appear to be regulated by blood flow,[56] the zebrafish genome encodes 2 copies of *miR-126*, one of which is induced by blood flow in the aortic arches, where it is required for Vegfa-dependent angiogenic remodeling.[56] In this case, Spred1 overexpression mimics the loss of miR-126, whereas reduction of Spred1 levels in *miR-126*–deficient embryos rescues aortic arch remodeling. Thus, Spred1, but not Pik3r2, is required for flow-mediated angiogenic remodeling of the aortic arches in the zebrafish. Further investigation using compound mouse knockouts deficient for *miR-126* and its targets will be insightful to determine the context-dependent requirements for Spred1 and Pik3r2 in mediating ERK and PI3K signaling downstream of Vegfa, during embryonic and postnatal angiogenesis.

MiR-221 Control of Vegfr-3 Signaling

Similar to miR-126, recent data suggest that miR-221 also acts to modulate Vegf receptor signaling through regulation of a PI3K regulatory subunit. Although previously characterized in other systems,[57] miR-221 was identified through deep-sequencing efforts as an endothelial-enriched miR in zebrafish embryos.[53] Knockdown of miR-221 does not affect initial blood vessel development but causes angiogenesis and lymphatic defects[53] that are remarkably similar to loss of Vegfr-3, a receptor for the Vegfc ligand.[29] Furthermore, epistasis studies suggest that miR-221 acts within the Vegfc/Vegfr-3 signaling pathway, and in parallel to Vegfa/Vegfr-2. Mosaic analysis revealed that miR-221–deficient cells do not contribute well to the tip cell position in developing angiogenic sprouts. By contrast, overexpression of miR-221 induces tip cell behaviors, such as enhanced proliferation and migration. The effect of miR-221 on cell proliferation appears to be largely mediated by repression of cyclin-dependent kinase inhibitor 1B (Cdkn1b), and miR-221 also targets Pik3r1, which was previously shown to interact with Vegfr-3.[58] Both reduction and overexpression of Pik3r1 result in inhibition of angiogenesis,[53] suggesting that precisely tuned levels of this regulatory subunit are required for optimal PI3K output.

These studies suggest that a common mechanism by which miRs regulate intracellular growth factor signaling is by precisely tuning the level of PI3K regulatory subunits. Studies showing that Pik3r1 and Pik3r2 are targeted by several different miRs during growth factor signaling in non-EC types further suggest that this may be a general theme.[59–61] Interestingly, in these contexts, miR-mediated repression of the regulatory subunit is associated with reduced proliferation and apoptosis. By contrast, both miR-126 and miR-221 promote endothelial growth and angiogenesis suggesting that they fine-tune, rather than simply inhibit, PI3K activity to elicit context-dependent Vegf signaling outputs. Moreover, these miRs appear to control output through 2 distinct receptors: miR-126 regulates Vegfr-2, whereas miR-221 regulates Vegfr-3 signaling. These studies would further imply that these receptors each use different PI3K signaling complexes. In general, these miRs could provide a mechanism by which a cell may respond differentially to, or appropriately integrate, signaling in response to Vegfa and Vegfc.

MiR-132 Control of Ras Activity

Ras is a key regulator that acts downstream of the Vegf receptor to mediate activation of the mitogen-activated protein kinase/ERK pathway, and recent findings have suggested that Ras activity in ECs is controlled by miR-132 during tumor angiogenesis. Activity of Ras is controlled by opposing GTPase-activating proteins and GTP exchange factors that dictate whether Ras is in a GTP-associated active form, or a GDP-associated inactive form. MiR-132 was found to be highly expressed in tumor ECs, but not in normal ECs, and its induction is driven by angiogenic factors such as Vegf and fibroblast growth factor 2.[62] Silencing of miR-132 expression decreases the miR-132 target, p120RasGAP[62] (which negatively regulates Ras, and contributes to vascular development and remodeling).[63] Induction of miR-132 expression in ECs may act as an angiogenic switch during tumor neovascularization, and antagonism of miR-132 markedly decreases tumor angiogenesis in mouse models.[62] Furthermore, the role of miR-132 appears to be EC-specific, as its inhibition has little effect on the expression of p120RasGAP in other cell types.[62]

MiRs Regulate Signaling Pathways That Cross-Talk With Vegfr-2

Cross-talk with several signaling pathways also contributes to the precise modulation of Vegf signal output to govern appropriate EC responses.[1] Several recent studies have revealed that these pathways are also targeted by miRs in ECs (Figure).

These include components of the Slit/Roundabout (Robo) pathway, which are widely known for their roles as repulsive cues during neuronal guidance,[64,65] the Notch pathway, which plays a central role in switching off angiogenic behaviors induced by Vegf signaling,[29] as well as integrins, which can modulate Vegfr-2 signaling.[66]

MiR-218 Control of Slit/Robo Signaling

The Slit family of ligands (Slit1–3) bind to their cognate Robo receptors (Robo1–4) to control cell migratory behaviors. Although this pathway is best known for its regulation of neuronal guidance,[64,65] this ligand–receptor system also impinges on the Vegf signaling pathway in ECs. For example, Slit2 can activate Robo1 and Robo4 receptors, which are expressed on ECs,[67] to elicit divergent EC responses. Slit2 activates a Robo4-dependent signaling pathway in ECs that represses Vegf signaling by inhibiting the small GTPase, Arf6.[68,69] In contrast, Robo1 potentiates Vegf signaling by enhancing phosphorylation of Vegfr-2.[70] Recently, a miR family (miR-218) was found to be intronically encoded in the *Slit2* and *Slit3* genes.[70,71] Intriguingly, Slit-encoded miR-218 targets the Slit receptor, Robo1, as well as components of the Hspg biosynthetic pathway, and negatively regulates angiogenic responses in ECs.[70,71] Hspg have previously been shown to enhance Slit binding to Robo receptors,[72] and they also influence Vegf ligand–receptor interactions.[35] As miR-218 does not target Robo4, this miR may alter the activation of Robo1- versus Robo4-dependent pathways, which have opposing effects on Vegf signaling. Knockdown of miR-218 in the mouse retina results in defective angiogenesis,[71] and knockdown of miR-218 in zebrafish results in defects in the Vegf-dependent migration of the endocardium to the midline during the early stages of heart development,[70] illustrating the requirement of the Slit/miR-218/Robo1 pathway for normal cardiovascular development. The contribution of this pathway to pathological vascular growth remains to be determined.

Notch, MiRs, and Vegf

In sprouting blood vessels, Notch signaling plays a vital role in reducing the angiogenic response normally induced by Vegf.[29,73,74] In this case, Vegfa stimulates migration of leading tip cells from a preexisting blood vessel. During this process, Vegfa induces expression of the Notch ligand, Dll4, in the tip cell, which activates Notch signaling in adjacent cells, reducing their migration and proliferation. This provides an elegant mechanism to license a restricted number of cells to sprout from a preexisting vessel, and allows the growing sprout to maintain its connection to the patent blood vessel. Notch activation is thought to inhibit Vegfa-stimulated migration and proliferation by downregulating Vegfr-3 signaling,[29] and also induces the expression of soluble Vegfr-1,[75] which presumably acts as a sponge to bind surrounding Vegfa to prevent activation of Vegfr-2.[31]

Several studies suggest that cross-talk between Notch and Vegf signaling is further modulated by miRs. As mentioned above, miR-221 promotes tip cell behavior, in part through regulation of *Pik3r1* and modulation of signaling downstream of Vegfr-3.[53] Interestingly, Notch activation represses the expression of miR-221.[53] Also, excessive tip cell behaviors normally associated with loss of Notch signaling are blocked by loss of miR-221. Thus, Notch appears to affect the output of Vegf-3 signaling, in part through the regulation of miR-221 levels. Notch signaling components themselves have also been identified as miR targets during angiogenesis. In zebrafish embryos, miR-27b was found to repress the transcript encoding Dll4. Accordingly, miR-27b–deficient zebrafish embryos have reduced filopodia formation and impaired sprouting,[76] similar to embryos with activated Notch.[29] This is associated with increased expression of Dll4 and upregulation of Vegfr-1.[76] Interestingly, recent studies in cultured human cells and mice have shown that the miR-23/27/24 cluster is highly expressed in ECs and that miR-23 and miR-27 are capable of coordinately repressing Sprouty2,[77] a negative regulator of Raf1,[78] and Semaphorin 6A and D,[77,79] which inhibit Vegfr-2 signaling.[80] Thus, similar to miR-126 and miR-221, miR-27 can control the expression of multiple targets to modulate Vegf signaling output.

MiR-92a Control of Integrin Expression

ECs interact with several extracellular matrix proteins, including fibronectin, which can affect angiogenesis. For example, fibronectin can facilitate the migration of tip cells by enhancing Vegfr-2 signaling.[81] Fibronectins signal through several integrin proteins that are expressed on ECs. The importance of integrins for vascular growth is illustrated by the embryonic lethality and major vascular defects that are observed in integrin-α5 (*Itga5*) knockout mice.[82] Recently, miR-92a was shown to control angiogenesis through the targeting of *Itga5* in the endothelium.[83] Overexpression of miR-92a inhibits sprout formation and vascular network formation in vitro, and also represses vascular invasion of matrigel plugs loaded with angiogenic factors in vivo. Of particular interest, inhibition of miR-92a in mouse models of hind limb ischemia or myocardial infarction results in enhanced angiogenesis and tissue regeneration, strongly suggesting that miR-92a normally suppresses angiogenic signaling through its targeting of *ITGA5*.[83]

Emerging Areas of MiR Biology With Possible Implications for Vegf Signaling and Angiogenesis

In general, miRs are thought to act cell-autonomously to repress their target transcripts. The aforementioned examples of miR regulation have focused on this well-known role for miRs. However, recent findings suggest that miRs may act as paracrine or even endocrine factors. Furthermore, miR target transcripts in a given cell may function as sponges to titrate miR levels, rather than being functional targets themselves. Although little is known concerning the importance of these 2 new concepts in the context of Vegf signaling and angiogenesis, there are hints from recent studies that they will be relevant in this context. Therefore, we provide a brief overview of these new aspects of miR biology.

Several studies have now shown that miRs are abundant and relatively stable in circulation, suggesting that they may play a paracrine role in controlling gene expression.[84,85] MiRs have been associated with a variety of carriers, including lipid-encapsulated microvesicles, such as exosomes (50–100 nm), microparticles (0.1–1 μm), and apoptotic bodies (0.5–2 μm), as well as high-density lipoprotein and low-density lipoprotein complexes, and these carriers can deliver miRs to recipient cells.[86–88] Additionally, circulating Argonaute (Ago2)-containing

protein complexes that contain miRs may be the main carriers of circulating miRs,[89] but it is unclear whether these nonlipid-encapsulated complexes can be delivered to cells. Tumor cells can secrete miR-containing microvesicles that are taken up by ECs to alter EC phenotype, including their angiogenic properties.[90–92] It is currently not known whether secreted miRs contribute to vascular development, but this is an exciting prospect. A recent study has implied that levels of EC-enriched miRs, such as miR-126 and miR-92a, may be reduced in vascular diseases such as coronary artery disease,[93] but whether this is a cause or consequence of disease is not currently known. Further analyses in larger cohorts of age-matched patients will be necessary to clarify these initial findings.

Although the functional effects of miRs are typically thought to be manifested through the regulation of protein levels from a target transcript, recent observations suggest that many target transcripts may serve a different purpose. In this case, mRNAs containing miR-binding sites can affect the expression of other mRNAs in a protein coding-independent manner, via competition for the binding of miRs to their 3′-untranslated regions.[94,95] These RNAs have been named competing endogenous RNAs. Their function was first illustrated by the finding that a pseudogene for *PTEN*, *PTENP1*, can affect the expression of the *PTEN* gene by acting as a miR decoy.[96] Considering the central role that PTEN plays in controlling PI3K activity, an important output for Vegf signaling,[97] this finding will likely be relevant to vascular growth. It has subsequently been shown that multiple coding transcripts can control PTEN expression by titrating away miRs from the *PTEN* 3′-untranslated region.[98,99] A long noncoding RNA has also been shown to regulate muscle differentiation by acting as a competing endogenous RNA for miR-133.[100] It will be interesting to determine whether competing endogenous RNAs affect angiogenic signaling by competing for important proangiogenic or antiangiogenic miRs. Competing endogenous RNAs represent another layer of regulation that may impact the dynamic control of angiogenic signaling pathways.

Conclusion and Clinical Implications

It is evident that the Vegf signaling pathway is highly regulated at multiple levels. Recent studies demonstrate that miRs provide an additional layer of regulation by titrating the levels of proteins that are involved in the transduction of angiogenic signals. The general theme from these studies is that miRs are important nodes for controlling particular endothelial behaviors downstream of Vegf. Based on these observations, targeting miRs to manipulate subtle aspects of vascular growth in a clinical setting will be highly desirable. This is of particular importance, as many early angiogenesis inhibitors that bluntly target Vegf ligand–receptor interactions or Vegfr kinase activity result in side effects, such as hypertension and proteinuria.[101] MiRs can be targeted therapeutically,[102] and several findings from mouse models indicate that targeting miRs, including those implicated in Vegf signaling, may be useful in clinical settings where precise control of vascular growth is desired. For example, silencing miR-126 has been shown to impair neoangiogenesis after myocardial infarction[54] or hind limb ischemia.[103] Interestingly, the host transcript for *miR-126*, *Egfl7*, is highly upregulated in tumor endothelium,[104] but the role of miR-126 in tumor angiogenesis has not yet been addressed. Antagonism of miR-132[62] or miR-296,[44] which are upregulated in tumor endothelium, has promising inhibitory effects on tumor angiogeneis. In contrast, inhibiting miR-92a enhances vascular growth in the setting of myocardial infarction and hind limb ischemia.[83] These exciting results provide an impetus to further understand the role of miRs in modulating the signaling output of Vegf.

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None.

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