MINI REVIEW

# MicroRNA regulation of vascular function

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## Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that orchestrate genetic networks by modulating gene expression. Given their importance in vascular development, homeostasis and diseases, along with the technical feasibility in deploying their function *in vivo*, the so-called 'vascular miRNAs' have become key targets for therapeutic intervention. Herein, we have summarised the state-of-the-art on vascular miRNAs and we have discussed the role miRNA biogenesis and the extracellular vesicles (EVs) miRNA transport in vascular biology.

**Key Words**

- angiogenesis
- endothelium
- signalling

## Introduction to miRNAs in vascular biology

MicroRNAs (miRNAs) are a member of the small non-coding RNA family; they are ~22 nucleotides and are strong post-transcriptional regulator of gene expression. The specificity of miRNA targeting is defined by the complementarities between positions 2 and 8 of miRNA 5′-end (also termed the seed sequence), with generally the 3′-untranslated region of target mRNAs. The capacity of miRNAs to simultaneously inhibit many different mRNAs allows for an amplification of biological responses (1). The reference repository miRBase, currently holds information about 1917 human precursors and 2656 mature miRNAs (release 22) (2). In the last decade, the importance of miRNAs in vascular biology has consolidated, with miRNAs being established one of the major post-transcriptional regulatory elements in vascular biology. Among the miRNAs deposited in miRBase, 42 have been described as associated with endothelial cell function and angiogenesis (3). The ability of miRNAs to regulate vascularisation and to target several genes simultaneously makes them an extremely attractive target for therapeutic angiogenesis.

Analysis of miRNA transcription and target recognition have been extensively studied in the past years; however, two regulatory mechanisms will deserve specific attention in the future: regulation of miRNA biogenesis and intercellular transfer.

## Regulation of miRNA biogenesis in vascular biology

Most miRNAs are transcribed by RNA polymerase II as primary pri-miRNA transcripts, and undergo further processing by Drosha and Dicer nuclease complexes to produce miRNA duplexes 19–24 bp in length (4).

Research in the past few years has provided novel knowledge of miRNA biogenesis which have been also described in the vascular cells (Fig. 1). To date, there are not studies that describe aspects of miRNA biogenesis that are specific only for vascular cells.

Dicer-dependent control of miRNA expression plays an important role in the regulation of vascular function. Dicer is critical for embryonic (5) and postnatal angiogenesis (6) and regulates endothelial miRNA expression (7). Endothelial Dicer promotes endothelial inflammation and atherosclerosis in part by miR-103-mediated suppression of KLF4 (8). Interestingly, ageing-induced dysregulation of Dicer1-dependent

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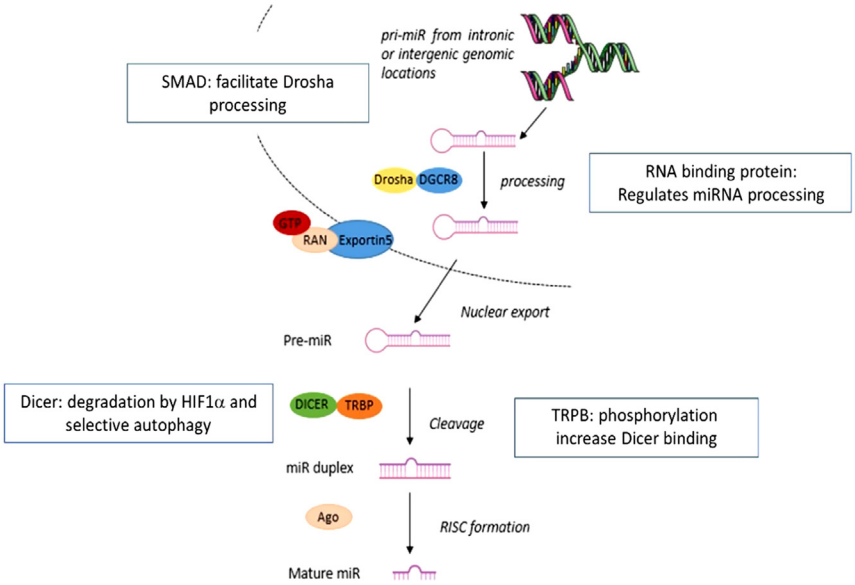


**Figure 1**
Regulation of miRNA biogenesis in vascular cells.

miRNA expression impairs angiogenic capacity of rat brain microvascular endothelial cells (ECs) (9).

Drosha, as well as Dicer, a key role in endothelial miRNA expression (7). Drosha-deficient zebrafish showed abnormalities in vascular development, and mice with an endothelial-specific knockout of Drosha exhibited disorganised, dilated vasculature and haemorrhage, which resemble the clinical presentations of HHT patients (10). Interestingly, the knockout of Drosha-regulating proteins DEADbox RNA helicases p68-p72 is embryonically lethal and knockout embryos display severe malformation of blood vessels (11). Argonaute 2 (AGO2)-associated miRNAs and mRNAs encoding cytoskeletal, contractile, adhesive and extracellular matrix (CAM) proteins have been identified (12). Inhibition of DROSHA or AGO2 promoted a contractile phenotype in endothelial and fibroblast cells *in vitro*, and increased tissue stiffness, contractility and extracellular matrix deposition in the zebrafish fin fold *in vivo* (12).

Crosstalk between different cellular pathways and miRNA biogenesis has been reported, and it is likely that many more such connections will be unravelled. For example, the regulatory potential of RNA-binding protein (RBPs) is tightly linked to miRNA biogenesis, and a broad layer of miRNA regulation by RBPs can be predicted (13). A recent paper demonstrated that post-transcriptional regulation of 14q32 miRNAs is mediated by the cold-inducible RBP (CIRBP) and hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta (HADHB) during vascular regeneration after ischaemia (14). Specifically, CIRBP and HADHB are upregulated after hind-limb ischaemia in mice and regulates the processing of miR-329 (14).

Furthermore, crosstalk between miRNA biogenesis and signal transduction through phosphorylation of miRNA-processing enzymes is emerging as an important regulatory principle in vascular disease. The regulation of miR-21 is crucial in vascular biology and SMADs are recruited to pri-mir-21 in a complex with the RNA helicase p68 and facilitate its DROSHA-mediated processing (15, 16). MAPK signalling affects miRNA biogenesis. TRBP is phosphorylated by the MAPK ERK, which increases the stability of the Dicer–TRBP complex and stimulates miRNA production (17). Additional stabilisation of TRBP is achieved through its phosphorylation by ribosomal protein S6 kinase (S6K), which is activated by ERK and mTOR, thereby integrating input from different signalling pathways. This also contributes to a pro-growth miRNA expression signature in lymphatic ECs and can be pharmacologically modulated (18).

Interestingly, also autophagy has been linked with miRNA biogenesis. DICER and AGO2 are targeted for degradation by the selective autophagy receptor NDP52. Autophagy establishes a checkpoint required for continued loading of miRNA into AGO2, and it is required for activity of miRNAs (19). Notably in the context of the vascular biology, a recent study showed HIF-1α binds directly Dicer and enhances its autophagy-mediated degradation by facilitating Dicer ubiquitination by the E3 ligase Parkin (20).

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## miRNA intercellular transfer and signalling via EVs

A revolutionary hypothesis, that extracellular miRNA can mediate cell–cell signalling via paracrine or even endocrine routes, emerged after several research groups found that substantial amounts of miRNA purified with extracellular vesicles (EVs) including microvesicles (MVs) and exosomes (21, 22). MVs are a heterogeneous population of EVs up to 2 µm in diameter called which are formed by budding and shedding of the cell membrane, a process that involves calcium-dependent signalling and enzyme activity. On the other hand, exosomes (50–100 nm) are a homogenous population of Evs, which are released from cells when multivesicular bodies (MVBs) fuse with the plasma membrane in a highly regulated process and release their contents (23).

Multiple follow-up publications demonstrated that miRNAs entrapped within various EVs can be transferred to recipient cells, alter gene expression and provoke functional effects (24). Moreover, several studies reported that miRNA content in exosomes was significantly different from that in parental cells, indicating that extracellular miRNAs may be selectively packed into the EVs (24). The extracellular miRNAs embedded in EVs are protected from nucleases activity (22, 25). Some studies report absence of AGO2 in the exosomes sub-group of EVs (26), whereas others report the presence of AGO2 protein (27). In this regard, RISC proteins in EVs could process precursor miRNAs (pre-miRNAs) into mature miRNAs, inducing the cell-independent miRNA biogenesis (28).

Recently a different theory on extracellular miRNAs has been proposed: the association of AGO protein-bound miRNAs with exosomes, microvesicles and apoptotic bodies could be explained by the well-known capacity of AGO2 to bind the membranes (29). It is possible that all extracellular miRNAs detected so far reside outside EVs, raising further dispute about the putative mechanisms of their export and penetration into target cells. Based on this hypothesis, the extracellular miRNAs have the potential to serve just as diagnostic tools for vascular disease with a limited transfer between the cells.

## Effects of intra- and extracellular miRNAs on vascular function

Recent studies have revealed important roles for miRNAs in regulating either physiological angiogenesis or post-ischaemic neovascularisation, particularly through the regulation of EC function (Table 1). The expression of miRNA in murine models of hind-limb ischaemia has been profiled showing that miR-100 (30), miR-424 (31) and miRNAs belong to miR-106b-25 cluster (32) control perfusion recovery and angiogenesis. Interestingly, inhibition of miRNAs belonging to the 14q32 locus has led to improvements in post-ischaemic blood flow *in vivo* (33). The regulatory effects on angiogenesis are not surprising within this miRNA cluster as it is known to target critical angiogenic factors including vascular endothelial growth factor A (VEGF-A) (33). The miR17-92 cluster is upregulated in hypoxic tissues and serves a proangiogenic role. The proangiogenic function of this cluster is associated with miR-18a and miR-19a (34), whereas the anti-angiogenic function with miR-92a. Systemic administration of miR-92a inhibitor restored vascularisation in a mouse model of hind-limb ischaemia and myocardial infarction (35). Recently, our work demonstrated that miR-503 has a prominent role in diabetes-induced impairment of post-ischaemic reparative neovascularisation (36, 37). miR-15a, miR-16 and miR-503 belong to the same family of miRNAs with overlapping targets because of common seed sequence (38). Transplantation of healthy circulating proangiogenic cells where miR-15a and 16 were inhibited, improved post-ischaemic blood flow recovery and vascularisation (39).

To better understand what miRNAs are involved in regulating the vasculature and what contribution they have in the disease pathology, numerous methods for detecting miRNAs and tools for overexpressing and knocking down miRNAs have been designed (40). One novel approach is to identify miRNAs involved in angiogenesis is high-content screening. In our own experiment, we used human miRNA mimic library to identify miRNAs important in the proliferation of ECs, a critical stage in the process of angiogenesis. Through this technique, we identified miRNA-26b as a positive regulator of endothelial cell proliferation and survival (41).

Endothelial MVs promote re-endothelialisation following endothelial injury in mice by stimulating endothelial migration and proliferation upon transfer of functional miR-126 to target endothelium, with subsequent downregulation of SPRED1 (42). We have demonstrated that NF-kB signalling induced miR-503 transcription and the shedding of endothelial MVs by triggering the expression of Rho kinase (36). miR-503-containing endothelial MVs are taken up by pericytes *in vivo*, thus increasing vessel permeability (36). Furthermore, miR-143 and miR-145 packaged in

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**Table 1** The effects of intra- and extracellular miRNAs on vascular function.

| miRNAs | Regulation | Localisation | Effect | Reference |
|---|---|---|---|---|
| miR-100 | Downregulation | Intracellular ECs | Increase vascularisation | (16) |
| miR-424 | Upregulation | Intracellular ECs | Increase vascularisation | (14) |
| miR-106b-25 cluster | Upregulation | Intracellular ECs | Increase vascularisation | (36) |
| 14q32 miRNA cluster | Downregulation | Intracellular ECs | Increase vascularisation | (45) |
| miR-18a | Upregulation | Intracellular ECs | Increase angiogenesis | (10) |
| miR-19a | Upregulation | Intracellular ECs | Increase angiogenesis | (10) |
| miR-92a | Downregulation | Intracellular ECs | Increase vascularisation | (3) |
| miR-15a-16 | Downregulation | Intracellular circulating proangiogenic cells | Increase vascularisation | (37) |
| miR-26b | Upregulation | Intracellular ECs | Increase vascularisation | (30) |
| miR-503 | Downregulation | Extracellular from ECs to pericytes | Increase vessels permeability | (5) |
| miR-126 | Upregulation | Extracellular from ECs to Ecs | Re-endothelisation | (21) |
| miR-143-145 | Upregulation | Extracellular from ECs to smooth muscle cells | Atheroprotective | (19) |
| miR-132 | Upregulation | Extracellular from pericytes to ECs | Increase vascularisation | (24) |
| miR-10a | Upregulation | Extracellular from monocytes to ECs | Anti-inflammatory | (33) |
| Let-7b | Upregulation | Extracellular from PF to Ecs | Increase vascularisation | (2) |

endothelial EVs released under shear stress are taken up by smooth muscle cells, where they downregulate target genes, inducing atheroprotective effects (43). On the other hand, pericyte-derived miR-132 is taken up by ECs, resulting in a higher proangiogenic capacity (44). A recent study showed that several anti-inflammatory microRNAs including miR-10a were transferred to monocytic cells from EC-EVs and could repress inflammatory signalling through the targeting of several components of the NF-κB pathway (45). Finally, analysis of pericardial fluid (PF) showed that exosomes are enriched in proangiogenic miRNAs (46). Delivery of PF exosomes in a mouse model of hind-limb ischaemia improved post-ischaemic blood flow recovery and angiogenesis in mice (46).

## Conclusions

There is no disputing that miRNAs have a huge influence on angiogenesis and vascular homeostasis and have the potential to be the next generation of vascular therapeutics, however, several technical challenges remain before this can become a reality and incorporated into daily treatments for vascular disease. Analysis of miRNA biogenesis in vascular biology could reveal new mechanisms to regulate miRNA abundance. Importantly, selective pharmacologic regulation of the mechanisms involved in post-transcriptional regulation of miRNA biogenesis, such as inhibition of ERK or S6 kinases, could provide a foundation for therapeutic intervention in cardiovascular diseases underpinned by deregulated miRNA levels. Finally, the understanding of the fundamental roles of each type of EVs have in the vessels and the discovery of the comprehensive mechanism behind the sorting of miRNAs inside the EVs are the key steps to develop EVs as a tool for efficient therapeutic angiogenesis in vascular disease.

**Declaration of interest**
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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