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SPOTLIGHT REVIEW

MicroRNAs in flow-dependent vascular remodelling

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Abstract Changes in haemodynamic forces in the vascular system result in an altered expression of miRs, which play important gene-regulatory roles by pairing to the mRNAs of protein-coding genes to fine-tune post-transcriptional repression. The development and structure of blood vessels are highly adapted to haemodynamic forces, such as shear stress, cyclic stretch, and circumferential wall stress, generated by the conductance of blood. Thus, fluctuations in shear stress contribute to miR-regulated differential gene expression in endothelial cells (ECs), which is essential for maintenance of vascular physiology. Several microRNAs have been identified that are induced by high shear stress mediating an atheroprotective role, such as miR-10a, miR-19a, miR-23b, miR-101, and miR-143/145. While changes in the expression profile of miR-21 and miR-92a by high shear stress are associated with an atheroprotective function, low shear stress-induced expression of miR-21, miR-92a, and miR-663 results in a pathological EC phenotype. MiR-155 fulfils pleiotropic functions in different regions of vasculature, when exposed to different modes of shear stress. Thus, changes in shear stress result in differential expression of numerous miRs, triggering the balance between susceptibility and resistance to cardiovascular diseases. Further elucidating the regulation of miRs by flow may allow future clinical applications of miRs as diagnostic and therapeutic tools.

Keywords Vascular remodelling • Shear stress • microRNA • Atherosclerosis

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1. Introduction

The development and structure of blood vessels are highly adapted to haemodynamic forces, such as tangential shear stress and circumferential wall stress, generated by the pulsatile blood flow.[1] In the adult vasculature, the proper differentiation of the main cell types in the vessel wall, endothelial cells (ECs) and smooth muscle cells (SMCs), are closely linked to the local haemodynamic cues.[2] ECs are primarily exposed to the laminar flow, which generates a mean wall shear stress of ~15 dyn/cm^2 in the arterial system, although the actual values may considerably vary in different locations.[3] In this range, shear stress promotes the differentiation of embryonic stem cells into ECs, stimulates the antithrombotic activity of ECs, and inhibits EC proliferation.[4–6] In contrast, reduction in shear stress (e.g. by oscillatory flow) below a certain threshold increases the turnover of ECs due to enhanced apoptosis and proliferation and induces a pro-inflammatory phenotype characterized by adhesiveness of leucocytes.[4,5] Accordingly, low levels of shear stress due to disturbed flow are typically found in regions of the arterial tree, which are highly susceptible for atherosclerosis, such as branching points or the outer curvature of the aortic arch.[6]

In addition to shear stress, the transmural pressure difference induced by the blood pressure in the vascular lumen mechanically strains the vessel wall. Due to the pulsatile changes of the blood pressure, the arterial wall is stretched by 2–15% during the cardiac cycle.[4] This cyclic mechanical stretch, which is tangential to the direction of the blood flow, primarily affects medial SMCs by promoting a contractile phenotype.[7,8] The contractility of SMCs is of crucial importance to establish the circumferential wall tension, which counteracts the transmural pressure difference and thereby regulates blood pressure and blood flow. Moreover, mechanical stretch modulates many functional features of SMCs, such as proliferation, apoptosis, and migration, which are closely related to vascular remodelling.[8] However, the degree of mechanical stretch appears to determine the type of response in SMCs. Physiologic strain maintains a quiescent and contractile phenotype, whereas static conditions and supra-physiologic stretch trigger a maladaptive response characterized by increased proliferation and enhanced synthetic capacity.[7,9] The mechanical stretch threshold that triggers SMC proliferation is much lower in venous than in arterial SMCs.[10] Therefore, venous bypass grafts are prone to neointima formation following arterialization. Moreover, increased wall tension in resistance arteries due to arterial hypertension promotes thickening of the media mainly by the development of hypertrophic, polyploid SMCs.[9,11,12] In addition to a reduced shear stress, mechanical stretch to the vessel wall is elevated at predilection sites of atherosclerosis and lowering circumferential

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wall tension can limit atherosclerosis, indicating that both haemodynamic factors play a role in atherogenesis.[13,14]

Together, shear stress and circumferential wall strain are crucial haemodynamic factors in the development of the vascular wall and in the differentiation of its main cell types, ECs and SMCs. However, disturbances of blood flow or blood pressure, for instance in regions of the arterial tree like branching points or the lower curvature of the aortic arch, can contribute to vascular diseases, such as atherosclerosis, by deranging the phenotype of vascular cells.

MicroRNAs (miRNAs) are small, non-coding, single-stranded RNAs, which regulate gene expression by post-transcriptional repression and thus modulate differentiation and cell fate decisions.[15–18] Accordingly, a network of microRNAs has been identified that controls the response of vascular cells to haemodynamic stress.

2. miRNAs regulate gene expression

In the human genome, more than 1000 miRs have been annotated up to now and their expression considerably varies in different cell types and during differentiation.[19] The transcription of intergenic miRs genes is regulated by transcription factors and many examples have been described that miRs regulate their own transcription by negative or positive feedback loops with specific transcription factors.[20,21] Notably, some miRs are encoded by introns of the protein-coding genes and thus their expression is regulated with the expression of the respective host genes, by generating co-transcriptional units of mRNA and miR.[19]

Mature miRs are generated from the primary transcripts by two subsequent steps of endonucleolytic cleavage by the ribonuclease enzymes Drosha and Dicer.[22] Following transcription by RNA polymerase II, the several kilobases long, primary miR (pri-miR) is processed by Drosha into a ~70 nt long precursor miR (pre-miR). After translocation into the cytoplasm by Exportin-5 (XPO5) in complex with the cofactor Ran-GTP, the pre-miR undergoes a second step of processing catalysed by Dicer, a member of the RNase III family.[22] Dicer complexes the Tar RNA binding protein (TRBP) which cleaves the pre-miR into the ~22 nt long mature miR duplex and mediates loading of one strand of the miR duplex, called guide strand, to Argonaut proteins. The second miR strand, the passenger strand, dissociates from the Argonaut protein by unwinding and is subsequently degraded.[23,24] However, miR passenger strands are not always degraded and both strands may co-accumulate in a tissue-dependent manner.[25] The abundance of the target mRNA expression for each miR strand modulates mature miR degradation and may thus contribute to tissue-dependent strand selection.[26] One member of the Argonaut protein family bind to GW182, which represents a glycine/tryptophan repeat protein. It has been shown that the N-terminal GW repeats of GW182 interact with the PIWI domain of Ago1, thereby forming the core protein component of the miR-induced silencing complex (miRISC) and protecting the miRs from degradation.[27] This complex presents the nucleotides 2–8 from the 5′ end of the miR (the seed region) for target sequence recognition, which is essential for miR-mediated gene silencing. Annealing of the seed region with the complementary sequence primarily in the 3′-UTR of the target mRNA is often sufficient for target recognition.[16] However, this short target recognition element may be present in various mRNAs and thus a single miR can regulate the expression of a large number of target mRNAs.[28] The miRISC has been reported to inhibit cap-dependent translation at the initiation phase by interfering with ribosome recruitment[29] and at post-initiation steps by inhibiting ribosome elongation.[30,31] Moreover, the miRISC also mediates deadenylation with subsequent decapping and degradation of the target mRNAs.[31,32] Thus, the target mRNA repression by miRs is mediated by inhibition of translation and/or mRNA degradation.[31]

3. miRs in the vascular development

During vertebrate embryogenesis, vascular patterning is initially guided by conserved genetic pathways that act before circulation. Subsequently, ECs must incorporate the mechanosensory stimulus of blood flow with these early signals to shape the embryonic vascular system.[33] Few details are known about how these signals are integrated during development; however, there is accumulating evidence that during vascular remodelling fluctuations in shear stress also affect the expression of miRs in ECs.[34–36]

Accordingly, interference with the miR biogenesis by deletion of Drosha, Argonaute 2 (Ago2), or Dicer demonstrated an essential role of miRs in cardiovascular development. Zebrafish Dicer-null embryos exhibited severe defects most prominently in gastrulation, brain morphogenesis, and cardiac development in association with a disrupted blood circulation.[37,38] Loss of the first two exons of the Dicer gene ($Dicer^{ex1/2}$) in mice leads to lethality in early embryogenesis due to defective blood vessel formation and maintenance.[39] The defects observed in $Dicer^{ex1/2}$ embryos were associated with aberrant expression of vascular endothelial (VE) growth factor (VEGF), its receptors VEGFR1 and VEGFR2 as well as the putative angiopoetin-2 receptor (Tie-1), suggesting that Dicer fulfils a central role in embryonic angiogenesis, most likely by processing miRs that regulate the expression levels of key angiogenic regulators.[38,39] Correspondingly, the knockdown of Dicer in ECs *in vitro* altered expression patterns of proteins that play essential roles in EC biology and angiogenic responses, such as Tie-2/TEK, VEGFR2, endothelial nitric oxide synthase (eNOS), interleukin-8, and angiopoietin-like 4 (ANGPTL4).[40] Moreover, Dicer silencing in ECs increased the expression of thrombospondin-1 (Tsp1),[41,42] a multidomain matrixglycoprotein that has been shown to be an endogenous inhibitor of angiogenesis, which may explain, in part, the anti-angiogenic phenotypes observed *in vitro*.[38] Interestingly, knockouts of single miRs in mice are normally not associated with a lethal phenotype, most probably because special groups of miRs perform at least partially redundant roles.[43]

Shear stress induces pronounced changes in gene expression of ECs.[44–46] The effects of flow on the transcription of genes that are crucial for vascular homeostasis, such as platelet-derived growth factor B (PDGFB), nitric oxide synthase 3 (NOS3), and platelet endothelial cell adhesion molecule 1 (PECAM1), are regulated by shear stress-responsive elements (SSREs) within their promoter regions.[47] Fluid shear stress regulates binding of mechanosensitive transcription factors, such as nuclear factor kappa B (NF-κB) and early growth response 1, to SSREs in ECs, where they regulate blood vessel homeostasis. ECs also sense shear stress through a mechanosensory cell–cell adhesion complex containing PECAM-1, VE-cadherin, and vascular endothelial growth factor 2 (VEGFR2). Accordingly, flow-mediated mechanical distortion of the ECs activates NF-κB through the integrin-p38 MAPK signalling cascade.[47–49]

In addition, shear stress also controls the expression of various miRs, which are involved in the regulation of morphological and functional changes of ECs in response to flow. However, mechanisms concerning miR regulation by shear stress are only fragmentary understood.

To obtain a deeper understanding, several miR expression profiling studies in ECs have been performed to identify miR expression patterns

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which are regulated by shear stress.[50–55] A subset of miRs has been identified that is induced by high shear stress mediating an atheroprotective role, including miR-10a, miR-19a, miR-23b, miR-101, miR-126, and miR-143/145. In contrast, low shear stress-induced expression of miR21a, miR-92a, and miR-663 results in an atheroprone EC phenotype. Based on these findings, this review article will discuss the regulation and the (patho)physiological functions of shear stress-regulated miRs in the context of vascular biology.

4. miR-10a represses the inflammatory response of ECs

The mammalian miR-10 family is evolutionary ancient, highly conserved, and consists of miR-10a and miR-10b, which are encoded within the *HOX* gene cluster.[56] *HOX* genes encode evolutionary conserved transcription factors that are crucial for development.[57] Moreover, there is accumulating evidence for a close interplay between *HOX* gene regulation and the expression levels of miR-10 family members.[56] Due to the fact that miR-10a and miR-10b differ by only one base in the seed region, they are therefore predicted to share common targets. In this context, miR-10a/b have been shown to target several *HOX* transcripts during different developmental processes.[58–60] Up to now, p65 and TWIST are the only reported direct regulators of miR-10a and miR-10b, respectively.[61,62]

Interestingly, a recent *in vitro* study identified miR-10a as a flow-responsive induced miR in ECs,[50] which could be confirmed by Hergenreider *et al.*[63] In an *in vivo* study, the potential contributions of regulatory miRs within regions of susceptibility to atherosclerosis were investigated by artery site-specific miR profiling in adult swine.[55] This approach revealed that the expression of endothelial miR-10a was lower in the atherosusceptible regions of the inner aortic arch and aorta–renal branches than in other regions. mRNA microarray analysis of the miR-10a knockdown in cultured human aortic ECs identified IκB/NF-κB-mediated inflammation as the major pathway which was up-regulated after RNAi against miR-10a.[55] This indicates that miR-10a mediates an anti-inflammatory effect. It is of special interest that two key regulators of IκBα degradation, mitogen-activated kinase kinase kinase 7 (MAP3K7; TAK1) and β-transducin repeat-containing gene (*βTRC*),[64] contain a highly conserved miR-10a binding site in their 3′-UTR, which mediate miR-10a suppression of these pro-inflammatory molecules.[55] Thus, the induction of miR-10a upon shear stress contributes to the repression of pro-inflammatory endothelial phenotypes in atherosusceptible regions *in vivo* (*Figure 1A*).

5. miRNA-21 mediates different effects on ECs upon high and low shear stress

miR-21 plays a fundamental role in the regulation of (patho)physiological processes, including development, cancer, inflammation, and cardiovascular diseases.[65] The gene of pri-miR-21 is located in the intronic region of the TMEM49 gene, where it is independently transcribed by its own promoter.[66] Early studies of miR-21 regulation revealed that the pairs IL6/STAT3 and PMA/AP-1 were effective inducers of miR-21 expression.[67–69] Moreover, additional reports have unravelled multiple alternative regulators for miR-21, such as induction through Ras, ERK1/2, EGFR, and oestrogen receptor.[70–73]

In a recent study performed on human umbilical vein endothelial cells (HUVECs) exposed to prolonged unidirectional shear (USS) stress, 13 miRs were identified whose expression was significantly up-regulated.[52] The miR with the greatest change was miR-21. PTEN, a known target of miR-21, was down-regulated in HUVECs exposed to USS or transfected with pre-miR-21. HUVECs overexpressing miR-21 had decreased apoptosis and increased eNOS phosphorylation and nitric oxide (NO) production.[52] Moreover, miR-21 decrease EC migration via repression of RhoB.[74] Therefore, these data point to an atheroprotective function miR-21 (*Figure 1B*).

Another study revealed that oscillatory shear stress (OSS) induces the expression of miR-21 at the transcriptional level in cultured human umbilical vein ECs via an increased binding of c-Jun to the promoter region of miR-21.[75] OSS induction of miR-21 inhibited the translation of peroxisome proliferator-activated receptor-α, an important anti-inflammatory mediator in the vessel wall,[76,77] by 3′-UTR targeting. OSS and overexpression of miR-21 enhanced the expression of adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1), and monocyte chemotactic protein-1 and the consequential adhesion of monocytes to ECs. These findings suggest the presence of a positive feedback loop that enables the sustained induction of miR-21, thus contributing to the pro-inflammatory responses of vascular endothelium under OSS[75] (*Figure 2A*). It is noteworthy that the latter study is in contrast to the findings by Weber *et al.*[52] This discrepancy might be explainable by the fact that miR-21 is differentially regulated by diverging modes of shear stress or by additional factors beyond shear stress.[65]

6. miRNA-126 is essential for EC homeostasis

MiR-126 and its complement miR-126* are encoded by the *egfl7* gene within intron 7 in vertebrates,[78,79] which plays an important role in angiogenesis.[80] Expression analyses revealed abundant levels of miR-126 in highly vascularized tissues, and identified miR-126 as the only miRNA known to be specifically expressed in the EC lineage and haematopoietic progenitor cells.[79,81]

The absence of miR-126 during development increases vascular permeability and leakage, which is mainly due to up-regulation of the miR-126 targets SPRED-1 and PIK3R, which are both inhibitors of VEGF signalling.[79,81] Accordingly, loss of miR-126 *in vivo* caused impaired EC migration during vessel growth as well as collapsed vessel lumen and a compromised endothelial tube organization.[79,81] In a recent study in zebrafish, it has been shown that mature vessels express high levels of miR-126, which potentiate phosphoinositide-3-kinase (PI3K)-mediated angiopoetin 1 (Ang-1) signalling by reducing the levels of the PI3K regulatory subunit 2 (p85β). This regulatory circuit enhances the remodelling and stabilization effect of Ang-1.[82]

Notably, the passenger strand of pre-miR-126 is not completely degraded. As a consequence, miR-126 and miR-126* are both highly expressed.[81] KLF2 transcriptionally regulates miR-126 expression during development. While KLF2 induced by shear stress increase miR-126 levels in zebrafish,[34] no flow or KLF2-dependent up-regulation of miR-126 was detectable in human ECs.[54,63] miR-126 mediates up-regulation of CXCL12 by apoptotic bodies derived from ECs.[83] Targeting of RGS16 by miR-126 promotes a positive feedback loop in which increased activation of CXCR4 in the absence of RGS16 induces CXCL12 expression (*Figure 3*). Accordingly, the paracrine atheroprotective effect of apoptotic bodies derived from ECs is caused by

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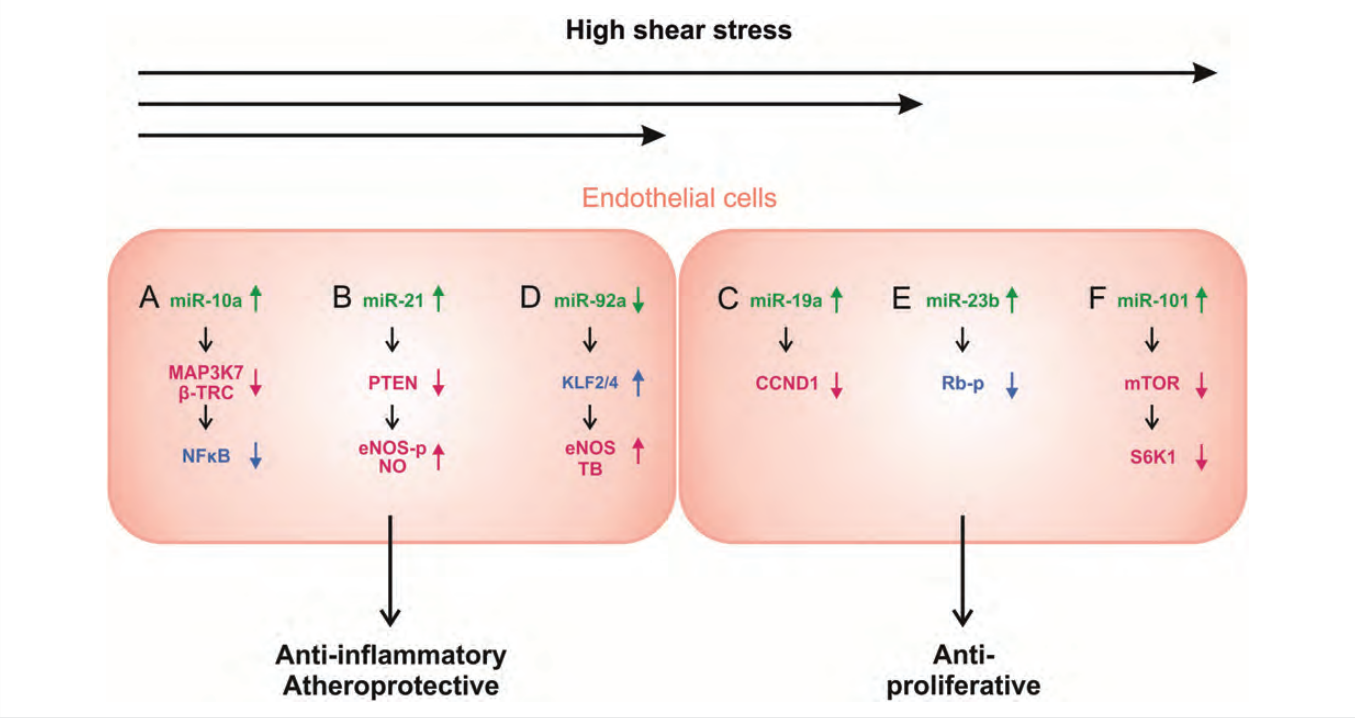

Figure 1 Shear stress-regulated miRs that support a physiological phenotype of ECs. While high shear stress (HSS) induces the up-regulation of miR-10a (*A*) and miR-21 (*B*), miR-92a (*D*) is repressed under these conditions leading to an anti-inflammatory and/or atheroprotective EC phenotype (left). Moreover, high shear stress comes along with an induction of miR-19a (*C*), miR-23b (*E*), and miR-101 (*F*) resulting in an anti-proliferative status of ECs. Colours: miRs, green; transcription factors or proteins that regulate transcription factor functions, blue; (general) mediators, red. β-TRC, β-transducin repeat-containing gene; CCND1, Cyclin D1; eNOS-p, phosphorylated endothelial nitric oxide synthase; HSS, high shear stress; KLF2/4, Krüppel-like factor 2 and 4; LSS; laminar shear stress; mTOR, mammalian target of rapamycin; MAP3K7, mitogen-activated kinase kinase kinase 7; NF-κB, nuclear factor κB; NO, nitric oxide; PTEN, phosphatase and tensin homolog; Rb-p, phosphorylated retinoblastoma; S6K1, ribosomal S6 kinase 1; TB, thrombomodulin.

increased expression of CXCL12 inducing the recruitment of cells to the endothelial lining.[83] Transfer of miR-126 enriched apoptotic bodies or even miR-126 itself into the ApoE knockout mice reduces the size of lesions, suggesting that the anti-atherosclerotic effect of ECs derived apoptotic bodies is at least partially performed by miR-126.[83,84] MiR-126 modestly suppresses endothelial VCAM-1 expression and leucocyte adhesion by activated ECs, which may contribute to its atheroprotective role.[85] Interestingly, circulating levels of miR-126 are substantially reduced in patients with coronary artery disease or insulin resistance/diabetes, which may be caused by defective packaging of miR-126 into endothelial microvesicles.[86,87] Recently, it has been demonstrated that miR-126 is substantially highly expressed in human $CD34^+$ compared with $CD34^-$ peripheral blood mononuclear cell (PBMC) subsets.[88] Notably, miR-126 was secreted by $CD34^-$ PBMC subsets, largely in microvesicles and exosomes. These vesicles were taken up by ECs which resulted in increased tube formation capacity. Notably, high-glucose treatment or diabetes reduced miR-126 levels of $CD34^+$ PBMCs, which was associated with impaired proangiogenic properties.[89] Moreover, it has been shown that miR-126 is expressed in HSC and early progenitors playing a pivotal role in restraining cell cycle progression of HSC *in vitro* and *in vivo*.[90] miR-126 knockdown resulted in HSC proliferation without inducing exhaustion leading to an expansion of mouse and human long-term repopulating HSC. Conversely, enforced miR-126 expression impaired cell cycle entry, which came along with a progressively reduced haematopoietic contribution. MiR-126 regulates multiple targets within the PI3K/AKT/GSK3β pathway, attenuating signal transduction in response to extrinsic signals. Thus, miR-126 sets a threshold for HSC activation and governs HSC pool size, demonstrating the importance of miRNA in the control of HSC function.[90] Concerning this background, it becomes feasible that also miR-126 containing microvesicles from other cell types are involved in the regulation of the HSC phenotype.

In summary, miR-126 plays a pivotal role by maintaining homeostasis of EC; however, shear stress regulation of miR-126 has only been demonstrated in zebrafish up to now (*Figure 3*).

7. Shear stress-induced expression changes of miR-17∼92a cluster members

The precursor transcript derived from the mir-17-92 gene contains six tandem stem-loop structures that generate six mature miRNAs, namely miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a.[91,92] These six miRNAs can be categorized according to their seed sequence into three separate miRNA families: the miR-17 family (miR-17, miR-18, miR-20), the miR-19 family (miR-19a, miR-19b), and the miR-92 family.[92] The transcription of miR-17-92 is directly activated by c-Myc. This finding is consistent with the functions of miR-17-92 in promoting

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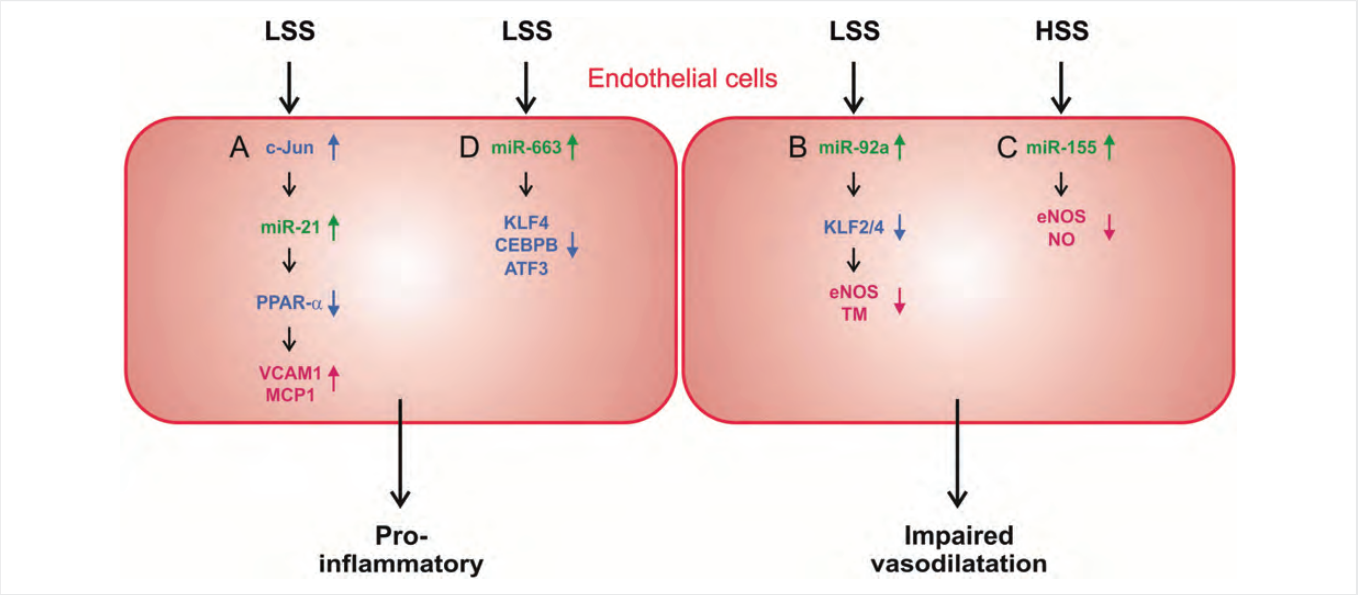

Figure 2 Shear stress-regulated miRs that mediate a pathophysiological phenotype of ECs. The induction of miR-21 via c-Jun (*A*) and miR-663 by low or disturbed shear stress (LSS) results in a pro-inflammatory EC phenotype (left). Enhanced expression of both miR-92a and miR-155 is associated with an impaired vasodilatation (right). Colours: miRs, green; transcription factors or proteins that regulate transcription factor functions, blue; (general) mediators, red. ATF3, activatin transcription factor 3; CEBPB, CCAAT/Enhancer-binding protein beta; c-Jun, jun avian sarcoma virus 17 oncogene homolog; eNOS, endothelial nitric oxide synthase; HSS, high shear stress; KLF2/4, Krüppel-like factor 2 and 4; KLF4, Krüppel-like factor 4; LSS, low shear stress; MCP1, monocyte chemotactic protein 1; NO, nitric oxide; PPARα, peroxisome proliferator-activated receptor α; TM, thrombomodulin; VCAM-1, vascular cell adhesion molecule-1.

proliferation in a variety of cell types, including cells of lymphoid, epithelial, and neural origin.[92] Moreover, the E2F family of transcription factors has been identified to bind to the promoter of miR-17-92 for regulating its transcription.[93,94] Collectively, these studies suggest a model, wherein c-Myc induces the proliferative machinery but establishes a threshold of E2F expression through repression by miR-17-92.[95]

Intriguingly, it has been recently reported that some members of the miR-17-19 cluster were regulated by changes in flow.[51] In this approach, miR-17, miR-20a, and miR-92a were down-regulated upon pulsative flow. In contrast, laminar shear stress induces miR-19a expression in ECs, which plays an important role in the flow-regulated expression of cyclin D1 and endothelial proliferation[50] (*Figure 1C*). Another study demonstrated that miR-92a is repressed by laminar flow and up-regulated by oscillatory flow.[96]

In this setting, it has been shown *in vivo* that miR-92a is highly expressed in ECs which were isolated from the atherosusceptible aortic arch when compared with atheroresistant regions,[97] indicating that miR-92a might impair endothelial functions during atherogenesis (*Figure 2B*).

Endothelial KLFs, particularly KLF4 and KLF2, are critical transcriptional regulators of endothelial homeostasis by establishing an anti-inflammatory, vasodilatory, and antithrombotic vascular phenotype.[98–101] *In silico* predictions and experimental validation demonstrated that endothelial miR-92a is an upstream regulator of KLF4 biogenesis and also plays a role in KLF2 expression.[96,97] Both KLFs are flow-induced transcription factors and their structural homology is closely correlated with functional similarity of regulation by miR-92a.[101]

Beside the regulation of KLF4 by shear stress, its expression is also induced by pro-inflammatory stimuli.[100] Overexpression of KLF4 in ECs activates expression of anti-inflammatory and antithrombotic genes, such as those that encode eNOS and thrombomodulin, whereas reduction in KLF4 levels increases tumour necrosis factor-α-induced expression of VCAM-1 and tissue factor.[100] KLF2 is restricted to the endothelium of healthy aorta, in regions exposed to laminar shear stress.[45] In contrast, KLF2 expression is decreased or absent in vessel regions which are exposed to non-laminar shear stress, such as bifurcations of the aorta to the iliac and carotid arteries, which are highly susceptible to atherosclerosis.[46] It is well established that KLF2 induces eNOS expression and mediates pleiotropic atheroprotective effects.[102] Moreover, endothelial expression of KLF2 mediates paracrine effects on cocultured SMCs by reducing their migration capacity.[103] This is similar to the failure of SMCs to properly migrate to the developing aorta in KLF2$^{-/-}$ mouse embryos.[104] Overexpression of miR-92a inhibits the expression of eNOS and administration of miR-92a into mice decreases the expression of KLF2 and eNOS in the arteries.[96] The reciprocal expression of endothelial miR-92a and KLF4/2 in arterial regions exposed to locally disturbed blood flow *in vivo* is consistent with underlying haemodynamic mechanisms, including the flow-sensitive nature of KLF4 and KLF2.[45,105] Wu *et al.*[96] recently reported that an atheroprone flow waveform increased not only the level of endothelial miR-92a but also the association of miR-92a and KLF2 mRNA with both Ago1 and Ago2 proteins that are associated with the RNA-induced silencing complex, providing molecular evidence for the regulation of endothelial KLFs by miR-92a. Consistent with these observations, mouse carotid arteries receiving miR-92a precursor exhibited impaired vasodilatory response to flow. Taken these findings together, atheroprotective flow patterns decrease the level of miR-92a, which in turn increases KLF4/2 expression to maintain endothelial homeostasis[96] (*Figure 1D*).

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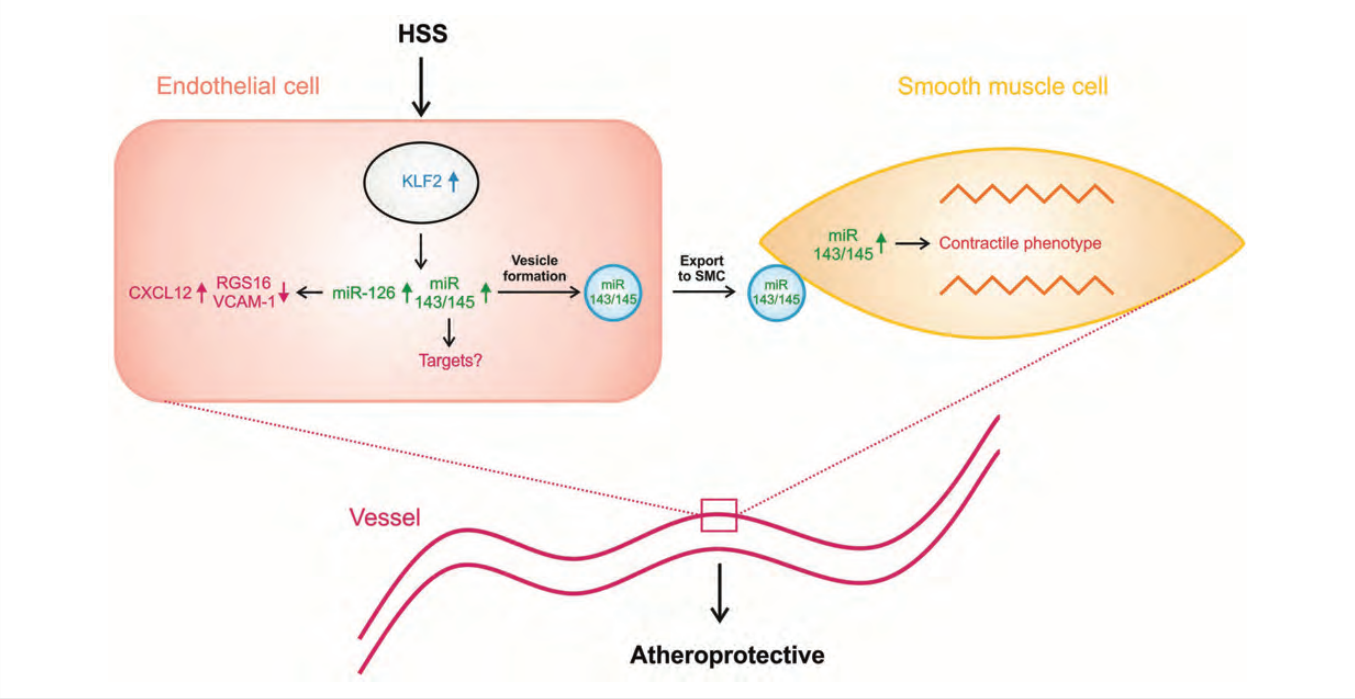

Figure 3 High shear stress induces the expression of KLF2. Mir-126 is up-regulated by KLF2, thereby repressing RGS16 and VCAM-1. This results in an induction of CXCL12 which mediates an atheroprotective function (left). However, the regulation of miR-126 by shear stress/KLF2 has only been demonstrated in zebrafish. MiR-143/145 are direct targets of KLF2 in ECs and are up-regulated by laminar shear stress. The downstream targets of miR-143/145 in ECs have not identified up to now. However, laminar shear stress triggers the release of EC-derived microvesicles that are highly enriched in miR-143/145, both via KLF2 (left). These EC microvesicles transfer miR-143/145 to SMCs. By repressing proliferation and enhancing differentiation of SMCs, miR-143/145 communicate atheroprotective functions (right). Colours: miRs, green; transcription factors or proteins that regulate transcription factor functions, blue; (general) mediators, red. CXCL12, Chemokine CXC motif ligand 12; KLF2, Krüppel-like factor 2; RGS16, regulator of G-protein signalling 16; VCAM-1, vascular cell adhesion molecule-1.

8. miR-23∼27∼24 cluster members regulate cell cycle progression and angiogenesis

The mouse miR-23a∼27a∼24-2 cluster is located on an intergenic region of chromosome 8, whereas the miR-23b∼27b∼24-1 cluster is located in intron 4 of an alanine aminopeptidase gene on chromosome 13107. All cluster members are highly expressed in vascularized tissues, such as the heart and lung, and in ECs. The mature miRNA sequences of miR-23a/b, miR-27a/b, and miR-24 are conserved among vertebrate species.[106]

Several studies have shown that some members of these clusters are up-regulated by laminar flow, such as miR-23b, miR-27a, and miR-27b.[51,106] The induction of miR-23b by shear stress has been shown to inhibit cell cycle progression by blocking the phosphorylation of the retinoblastoma protein[51] (*Figure 1E*). MiR-27a and miR-27b are highly expressed in ECs. Moreover, miR-27b is down-regulated after Dicer or Drosha silencing and inhibition of miR-27b significantly reduced EC sprouting *in vitro*, pointing to a proangiogenic role of miR-27b.[41] In this context, it has been shown that miR-27a/b promotes angiogenesis by binding to the 3′-UTR of the angiogenesis inhibitor SEMA6A, which controls the repulsion of neighbouring ECs.[107] Moreover, miR-27a/b regulate angiogenesis and choroidal neovascularization by targeting the anti-angiogenic protein Sprouty.[106]

9. Induction of miR-143 and miR-145 by high shear stress is atheroprotective

miRNA-143/145 are highly expressed in SMCs and are encoded by a bicistronic transcript. miR-145 and miR-143 are co-transcribed in multipotent cardiac progenitors before becoming localized to SMCs, including neural crest stem cell-derived vascular SMCs.[108,109] The promoter of miRNA-143/145 is regulated by a transcription factor complex consisting of serum response factor and coactivators of the myocardin family.[108,109] Moreover, the miR-143/145 promoter contains a Smad consensus sequence and is activated in response to transforming growth factor- β (TGF-β) signalling.[110] Furthermore, miR-145 and miR-143 cooperatively targeted a network of transcription factors, including Klf4, myocardin, and Elk-1 to promote differentiation and repress proliferation of SMCs.[108] miR-143/145-deficient mice have a thinner medial layer in arteries and decreased blood pressure, indicating severely disturbed SMC homeostasis.[109,111,112] SMCs in miR-143/145$^{-/-}$ mice are partially dedifferentiated which comes along with a diminished expression of SMC-specific contractile proteins and actin stress fibres.[109,111,112]

Shear stress up-regulates miR-143/145 in ECs and triggers the release of EC-derived microvesicles that are highly enriched in miR-143/145, both via KLF2.[63] These EC microvesicles transfer miR-143/145 to SMCs, and

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treatment with microvesicles derived from KLF2-overexpressing ECs reduces atherosclerosis in a miR-143/145-dependent manner[63] (*Figure 3*).

It may be hypothesized from these *in vivo* studies that elevated levels of miR-143/145 promote a SMC phenotype which is atheroprotective by preventing dedifferentiation.[113] However, although the experimental findings *in vitro* are promising, the respective *in vivo* evidence for intercellular exchange of information through miRNA microvesicles is still lacking.[84]

10. MiR-155 regulates vascular response by haemodynamic forces

MiR-155 is encoded by B-cell Integration Cluster (BIC)[114] and is involved in various biological processes such as haematopoiesis, immunity, and inflammation.[115–118] MiR-155 plays an important role in cardiovascular disease and is expressed in myeloid cells, macrophages, vascular SMC, and ECs.[52,118–120]

MiR-155 expression is induced by prolonged high shear stress in HUVECs, suggesting that it may moderate the atheroprotective effects of high shear stress in arterial ECs.[52] Moreover, miR-155 modulates NO production in HUVECs by targeting endothelial NO synthase and thereby regulates endothelium-dependent vascular relaxation.[121] Thus miR-155 may regulate part of EC responses to shear stress (*Figure 2C*).

It has been recently shown that miR-155 expression is induced in response to acutely disturbed blood flow in the arteries of hyperlipidaemic mice. In the model of flow-induced atherosclerosis, miR-155 plays a pro-inflammatory role in the lesional macrophages and leads to exacerbated lesion formation.[120] However, miR-155 plays an anti-inflammatory role in hyperlipidaemic mice without induced disturbed blood flow and ultimately reduces atherosclerosis.[120,122] These data provide evidences for the potential role of miR-155 in response to altered blood flow and therefore progression of atherosclerosis.

Angiotensin II plays an important role in the regulation of blood pressure by activating contraction of vascular SMCs.[123,124] Most of the physiological effects of Ang II are mediated by Angiotensin II receptor, type 1 (AT1R).[125] MiR-155 is a post-transcriptional regulator of AT1R expression in human primary lung fibroblasts.[126] Impaired miR-155-mediated suppression of AT1R could be implicated in hypertension and cardiovascular diseases.[127] Taken together, these data suggest that miR-155, expressed in different vascular cells, regulates several targets in each cell type and thereby contribute in regulation of vascular response to haemodynamic forces directly or indirectly. However, the functional role of miR-155 in different regions of vasculature exposed to different shear stresses need to be further studied.

Just recently, it could be demonstrated by our group that during early atherosclerosis the most prominently up-regulated miRNA was miR-342-5p, which is expressed in lesional macrophages. Upon pro-inflammatory activation in macrophages *in vitro*, miR-342-5p promoted Nitric oxide synthase 2 (Nos2) expression in a miR-155-dependent manner by targeting Akt1, an inhibitor of miR-155 expression. Accordingly, the inhibition of miR-342-5p reduced atherosclerotic lesion formation and suppressed Akt1-dependent Nos2 expression in lesional macrophages.[128]

11. miR-101 inhibits cell cycle progression in ECs

In a recent study, it was reported that miR-101 is up-regulated in ECs exposed to laminar shear stress.[129] Bioinformatic analysis revealed mTOR as putative target of miR-101, containing a highly conserved and functional miR-101 binding site in its 3′-UTR.[129] Basically, cell cycle progression is promoted by mTOR through its downstream effector S6K1, which accelerates the G1/S transition.[130] Transfection of miR-101 led to a lower proportion of cells in S phase and a higher proportion in G0/G1 phase, indicating that miR-101 attenuated G1/S transition of cell cycle in ECs upon laminar shear stress[129] (*Figure 1F*).

12. miR-663 regulates KLF4 expression in ECs

By a microarray analysis of HUVECs that were exposed to OSS or LSS several miRs were identified that exhibited differential expression, in which miR-663 exhibited the most OSS-sensitive expression pattern.[54] Using a miR-663-specific inhibitor revealed that miR-663 specifically mediated OSS-induced monocyte adhesion to EC, pointing to a pro-inflammatory function of miR-663. In this study, several transcription factors (KLF4, CEBPB, ATF3) were identified targets of miR-663 under OSS conditions, which are involved in the regulation of numerous genes that are related to inflammatory responses. Interestingly, inhibiting miR-663 with miR-663-LNA restores KLF4 expression in ECs under OSS. This indicates that—in addition to miR-92a—OSS-induced miR-663 is critically involved in fine-tuning of KLF4 expression in ECs[54,100] (*Figure 2D*).

Conclusion

There is accumulating evidence that changes in shear stress affect the expression of miRs which mediate (patho)physiological changes of vascular cells during arterial remodelling and atherogenesis. This includes the control of the inflammatory response in ECs and macrophages as well as the regulation of the SMC phenotype following vascular injury. However, in many cases little knowledge exists with regard to the upstream signal transduction cascades controlling the expression of miRs under flow. Thus, the identification of shear stress-induced molecular pathways which affect miR expression in different vascular cell types is indispensable. Moreover, the ability to therapeutically manipulate miR expression and function through systemic or local delivery of miR inhibitors or mimics opens up a novel route for a new class of disease-modifying therapeutics based on miR biology. In this context, a deeper understanding of shear stress-regulated miRs will be beneficial for the development of miR-related therapeutic strategies in the treatment of cardiovascular diseases.

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