Atheroprotective mechanisms of shear stress-regulated microRNAs

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Summary
MicroRNAs (miRs) are small non-coding RNAs that control gene expression by inhibiting translation or inducing degradation of targeted mRNA. miRs play a crucial role in vascular homeostasis but also during pathophysiological processes. Functionally active endothelial cells maintain homeostasis of the vasculature and protect against cardiovascular disease. The mechanical activation of endothelial cells by laminar shear stress provides a potent atheroprotective effect and reduces endothelial inflammation and cell cycle progression. Laminar shear stress induces profound changes in gene expression and recently was shown to regulate various miRs. The down-regulation of miR-92a by shear stress enhances the expression of the endothelial nitric oxide synthase, whereas the up-regulation of miR-19a contributes to the shear-stress-induced inhibition of cell proliferation. In addition, members of the miR-23–27–24 cluster are increased and specifically miR-23b blocks cell cycle progression, whereas miR-27b was shown to reduce endothelial cell repulsive signals. Finally, increased miR-10 expression in atheroprotected regions reduced the inflammatory response of endothelial cells and increased endothelial miR-143/145 levels improved smooth muscle cells functions. Together, the regulation of miRs by shear stress contributes to the anti-inflammatory, cell cycle inhibitory and vasculo-protective effects in endothelial cells.

Keywords
Atherosclerosis, shear stress, angiogenesis and inhibitors, endothelial cells

Introduction
Endothelial cells (ECs) are subject to shear-induced mechanotransduction, which translates mechanical forces into signalling pathways that influence the phenotype of ECs. However, flow patterns can vary from relatively uniform laminar flow in unbranched portions of larger arteries (with a corresponding wall shear stress in the range of 10–70 dynes/cm²) to complex disturbed flow patterns near branch points, bifurcations, as well as sharp curvatures with generally low wall shear stress. Interestingly, in areas of disturbed flow and low shear stress earliest signs and subsequent progression of arteriosclerotic lesions occur, while the unbranched portions of arteries with undisturbed laminar flow are protected from atherogenesis (1). Beside the profound atheroprotective effects of laminar flow, shear stress is also considered as driving force for the remodelling of small vessels into arteries (a process termed arteriogenesis) as it occurs in response to ischaemia.

Different regimes of shear stress in the vasculature elicit unique biological responses in ECs (2). ECs that are exposed to turbulent flow and therefore low shear stress have a pro-inflammatory phenotype characterised by activation of pro-inflammatory transcription factors like nuclear factor (NF)κB and activator protein (AP)-1, and by expression of adhesion molecules on the surface. On the other hand, ECs that experience laminar shear stress are anti-inflammatory and express atheroprotective proteins like endothelial nitric oxide synthase (eNOS), which is induced by the transcription factor Krüppel-like factor 2 (KLF2) (3).

Posttranscriptional regulation by microRNAs
MicroRNAs (miRs) were first discovered in Caenorhabditis elegans (C. elegans) by Victor Ambros’ laboratory in 1993. Meanwhile about 2,000 human miRs are known, which control tissue homeostasis but can also mediate or contribute to various diseases (4, 5). The endogenous pathway of miR biogenesis involves the transcription of the primary miR by RNA polymerase II (6, 7). The primary miR transcripts are further processed in the nucleus by the RNase III enzyme Drosha and DGCR8/Pasha resulting in about 70 bp long precursor-miR. These precursors are then exported to the cytoplasm to be processed by the RNase III Dicer and finally the guide strand is preferentially incorporated into the Argonaute containing miR-induced silencing complex (miRISC), while the passenger strand (miRNA*) is usually but not always degraded. In mammals, miR functions are based on inhibition of effective mRNA translation of its target genes mediated by the imperfect base-pairing with the 3′-untranslated region (3′UTR) of target miRNAs. Alternative mechanisms include the direct induction of mRNA cleavage as well as diminished mRNA stability. As a conse-
sequence of the imperfect base-pairing between a miR and its target mRNA, bioinformatic estimates of target genes for an individual miR range from one to several hundred (8–12). Interestingly, miRs often target several members of the same pathway and as a consequence, even though the regulation of each individual target might be modest, the overall effect is much more profound. This effect was found for example for the inhibition of the fibrotic response by miR-29 (13), and the modulation of actin cytoskeletal dynamics by miR-145 (14).

MicroRNAs in vascular biology

First hints to an involvement of miRs in the vascular system came from studies showing that deletion of the miR processing enzyme Dicer in mice resulted in impaired blood vessel and yolk sac formation (15). Likewise, zebrafish Dicer mutant embryos display abnormal morphogenesis and vessel defects (16). The specific function of Dicer in ECs was confirmed by silencing Dicer expression in cultured ECs, which resulted in angiogenesis defects (17, 18). Hypomorphic deletion of Dicer by a Tie2-Cre deleter line in mice did not induce embryonic lethality but reduced neovascularisation in response to ischaemia (19).

Meanwhile various miRs were shown to control angiogenesis signaling, vascular inflammation and atherosclerosis as well as vessel remodelling and maturation (for review see [5, 20, 21]). This article will summarise the regulation and function of shear stress-regulated miRs in the vasculature.

Shear stress-regulated microRNAs

Exposure of ECs to shear stress induces profound changes in gene expression pattern (22, 23). Not surprisingly, shear stress therefore also controls the expression of various miRs, which in part contribute to the morphological and functional changes of ECs in response to flow. Several miR expression profiles have been generated to determine the changes in miR expression patterns induced by laminar shear stress for 12 hours (h) (24) or pulsatile laminar shear stress for 24 h (25, 26), while others compared the miR expression patterns induced by laminar versus oscillatory flow in human aortic valvular ECs (27) or human umbilical venous ECs (28). In addition, miR microarrays were performed with ECs isolated from atheroprotected and atherosusceptible sites of porcine vessels (29). Several of the regulated miRs meanwhile have been further functionally studied and were linked to the atheroprotective effects of shear stress (Fig. 1 and Table 1).

miR-17–92a cluster

The miR-17–92a cluster comprises several mature miRs namely miR-17, miR-18a, miR-19a and miR-19b, miR-20a and miR-92a. First evidence that the miR-17–92 cluster is regulated by shear stress was provided by Wang et al. showing that some miR-17–92a cluster members (miR-17, miR-19b, miR-20a, miR-92a) were down-regulated by 24 h pulsatile flow (25); however, validation experiments did not confirm the down-regulation of the most prominent regulated miR-19b (25). Subsequent studies, however, showed that another member of the miR-17–92 cluster miR-92a is down-regulated by laminar flow within 8–16 h and is up-regulated by oscillatory flow (30). These in vitro findings are consistent with studies in vivo showing that miR-92a is highly expressed in ECs isolated from the atherosusceptible aortic arch compared to atheroresistant regions (31) suggesting that miR-92a might impair endothelial functions and potentially contribute to atherogenesis. Indeed, miR-92a was shown to target the flow-induced transcription factors KLF2 (30) and KLF4 (31). KLF2 is well established to induce eNOS expression and exhibits various atheroprotective effects (3). Consistently, overexpression of miR-92a was shown to indirectly suppress eNOS expression (30). An indirect suppression of
miR-10a

An *in vivo* analysis of miRNA expression in large swine vessels reported that miR-10a was lower in athero-susceptible regions (29).
In vitro studies further delineated that inhibition of miR-10a enhanced the expression of a variety pro-inflammatory adhesion molecules (e.g. E-selectin, VCAM) and pro-inflammatory cytokines (e.g. IL-6, IL-8, MCP1) in ECs (29) suggesting that miR-10a exhibits an anti-inflammatory effect. These anti-inflammatory effects of miR-10a were mediated by a repression of mitogen-activated kinase kinase kinase 7 (MAP3K7) and β-transducin repeat-containing gene (βTRC), which control NFκB signalling (29). Given that miR-10a additionally targets the pro-apoptotic gene Bim1 in other cell types (42), one may speculate that miR-10a might exhibit an additional antiapoptotic effect.

miR-21

miR-21 is a stress-induced miR that controls tissue fibrosis and various other cellular responses. The regulation of miR-21 by flow is controversial. Zhou et al. reported that oscillatory shear stress augments miR-21 expression, which induces endothelial inflammation as measured by the induction of adhesion molecules and inflammatory cytokines (43). These pro-inflammatory effects of miR-21 were mediated by a repression of PPARγ (43). In contrast, Weber et al. showed that miR-21 is increased by laminar flow and reduces EC apoptosis and increases eNOS phosphorylation (26) indicating that miR-21 improves EC functions. The reason for these different findings might be that miR-21 is induced by various stimuli and both laminar and oscillating flow are known to activate ECs.

miR-126

One of the most highly expressed miR in ECs is miR-126. miR-126-null mice were found to be partially viable but their vessels are fragile and leaky and they show signs of impaired angiogenesis (44). Since miR-126 was shown to provide an atheroprotective effect (45), and high laminar shear stress is known to result in an atheroprotective state of the endothelium, the report that miR-126 is upregulated by flow and KLF2 in zebrafish embryos was intriguing (46). However, it was later realised that the zebrafish genome has two independent loci for miR-126 and the one that is regulated by flow is not conserved in mammals and consistently, miR-126 was not identified by miR expression profiles using human cells and no flow or KLF2-dependent induction was detectable in human ECs (28, 37).

miR-143/miR-145

Originally identified to control smooth muscle cell phenotype and contractile function (14, 47–49), miR-143 and miR-145 were recently found to be highly induced by laminar shear stress in ECs (37). MiR-143/145, which are transcribed as one primary intergenic cluster, control the expression of various proteins involved in actin cytoskeleton regulation in smooth muscle cells. Whether miR-143-145 contribute to the well-known actin cytoskeleton rearrangement upon shear stress stimulation or KLF2 overexpression in ECs remains to be established (50). Intriguingly, miR-143-145 in ECs are also exported in extracellular vesicles and taken up by smooth muscle cells, where miR-143-145 functionally regulate target gene expression (37).

Conclusion

Although many studies have described the effects of shear stress on the expression of miRs, still many more may be identified to play a role in the endothelial adaptations to shear stress. It also is not always well described which upstream signalling pathways relay shear stress sensing to altered miRNA expression and whether this occurs through the promoter of miR clusters or through processing of miRNAs. Many of the effects of shear stress are coordinated by KLF2 and for instance the miR-143/145 cluster is directly transcriptionally induced by KLF2, but for the majority of shear stress regulated miRNAs it has not been defined how they are induced or repressed. Furthermore, in vitro stimulation of cells of different origins with different shear stress regimes for various amounts of time will inherently lead to different miR expression profiles and it is therefore difficult to define one shear stress regulated miR signature. Moreover, in vivo ECs are not only exposed to shear stress, but are also influenced by signals from underlying smooth muscle cells and surrounding other endothelial cells, making extrapolations from in vitro experiments to the in vivo situation difficult. Nonetheless, shear stress has profound effects on EC phenotype and this is reflected by marked functional changes in the miR expression profile.

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Conflicts of interest

S. Dimmeler has served on the Advisory Board of Miragen. None of the other authors declares any conflicts of interest.

References