MicroRNA-126 in Atherosclerosis

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MicroRNAs (miRs) are small noncoding RNAs that post-transcriptionally control gene expression by binding to target mRNAs and thereby inducing translational inhibition or mRNA degradation. Most primary miRs are endogenously processed by endonucleases such as Drosha, and the resulting pre-miRs are then cleaved by an enzyme called Dicer to form ≈22-nucleotide duplexes. One strand of the duplex, the guide strand, is preferentially selected for entry in the silencing complex by argonaute-2, whereas the other strand, known as passenger strand or miRNA* strand, is typically degraded. However, in many cases, both strands are biologically active as miRs. In that case, both distinct mature miRs arise from the same pre-miR and are referred to as 5p and 3p (Figure).

Several miRs control the function of cardiovascular cells and contribute to tissue homeostasis or disease.1,2 For example, atherosclerosis is reduced by apoptotic body-mediated delivery of miR-126-5p.1 In contrast, miR-92a or miR-155 increases atherosclerotic lesion formation in mice models.3,4 A recent study by Schober et al6 now extends these findings and reports a distinct role of miR-126-5p versus miR-126-3p in atherosclerosis. Genetic deletion of miR-126, which depletes both miR-126-5p and miR-126-3p, increased atherosclerosis in apolipoprotein E–deficient mice. Analysis of target gene derepression showed that putative miR-126-5p targets were preferentially regulated in miR-126−/− mice, suggesting that miR-126-5p is the predominant atheroprotective regulator.6 This finding is consistent with the higher expression of miR-126-5p compared with miR-126-3p in endothelial cells, but was unexpected because miR-126-3p was previously found to target vascular adhesion molecules in endothelial cells and provide atheroprotective effects when systemically delivered.3,7 Perhaps these findings indicate that normal levels of miR-126-3p are relatively little involved in regulating endothelial inflammatory activation, because inhibition of miR-126-3p only mildly induces tumor necrosis factor-α–stimulated vascular cell adherence molecule 1, whereas overexpression or endogenous delivery via vesicles has profound anti-inflammatory and antiatherosclerotic effects. Subsequent studies in endothelial injury models confirmed that specific pharmacological inhibition of miR-126-5p but not miR-126-3p decreased endothelial repair and lesion size, whereas miR-126-5p mimics accelerated endothelial coverage.8

confirming the endothelial protective function of miR-126-5p. The authors additionally identified a new target of miR-126-5p, namely Delta-like 1 homolog (Dlk1), which controls endothelial cell proliferation and lesion formation.9 Dlk1 is a noncanonical inhibitor of Notch, and Notch inhibition blocked the proliferative effects of miR-126-5p overexpression in endothelial cells and enhanced endothelial repair in vivo. The link to Notch signaling was further confirmed by showing that Notch1 endothelial cell numbers were reduced in miR-126−/− mice, which is consistent with the increased expression of the Notch inhibitor Dlk1. Dlk1 was shown to impair angiogenesis by inhibiting endothelial cell proliferation in a Notch-dependent manner.8 Notch signaling is well known as crucial mediator of endothelial cell function during angiogenesis.9 Although others report that Notch signaling is proinflammatory,10 Schober and colleagues6 clearly demonstrate that Notch1 signaling in endothelial cells promotes proliferation through Hes5 (Hairy and enhancer of split 5) induction. In atherosclerosis models that are dependent on endothelial proliferation, like the denudation model and atherosclerosis formation at predilection sites, the proliferative effect of Notch signaling may prevail its proinflammatory effects. To further substantiate these claims and pave the way for translation into the clinical setting, it may be good to identify how human atherosclerosis depends on the balance between inflammation and proliferation of endothelial cells.

In addition to the identification of miR-126-5p and its target Dlk1 as major regulators of endothelial repair, the study by Schober et al6 addresses interesting conceptual aspects of flow-dependent regulation of endothelial cell homeostasis. By analyzing the focal nature of atherosclerotic lesion formation in miR-126−/− mice, they observed that miR-126 deficiency preferentially enhanced lesion formation in nonpredilection sites in which laminar flow is known to prevent lesion formation.6 Endothelial miR-126-5p was increased by laminar flow in vitro and in vivo. Previous studies in zebrafish showed that Krüppel-like factor 2 (Klf2) increased miR-126 expression11; however, other subsequent studies did not observe a shear or Klf2-induced upregulation of miR-126-3p in endothelial cells.12 These conflicting findings were attributed to the expression of 2 miR-126 genes in zebrafish, of which 1 is induced by Klf2, whereas only 1 apparently Klf2-insensitive miR-126 gene is present in mammals. The present study now shows that miR-126-5p but not miR-126-3p is increased by laminar flow, suggesting that laminar flow regulates argonaute-2–dependent selection of the guide strand and thereby specifically affects miR-126-5p processing or augments the stability of miR-126-5p. How this is regulated is not clear, but perhaps shear stress regulates one of the many post-translational modifications of argonaute-2,13 thereby altering strand selection. However, if miR-126-5p is increased by laminar shear stress and induces endothelial cell proliferation, how does this fit to the well-known antiproliferative effects of laminar flow?
Shear stress induces microRNA (miR)-126-5p, which provides atheroprotection via the inhibition of Delta-like 1 homolog (Dlk1). After processing by Dicer, miR-126-5p and miR-126-3p are loaded into RNA-induced silencing complexes. Through a mechanism yet unknown, specifically miR-126-5p levels are induced after cells are exposed to laminar shear stress. miR-126-5p inhibits the expression of Dlk1, an antiproliferative inhibitor of Notch, leading to enhanced endothelial proliferation and atheroprotection. VCAM 1 indicates vascular cell adhesion molecule 1.

One possible explanation is that in a healthy environment at nonpredilection sites, laminar flow limits endothelial apoptosis, and therefore, there is no need for the contact-inhibited endothelial cells to proliferate. In contrast, at predilection sites with ongoing inflammation and endothelial apoptosis, endothelial proliferation is required to maintain an endothelial monolayer. On downregulation of miR-126-5p by turbulent flow, the proliferative capacity of the endothelial cells is reduced, thereby limiting the adaptive response after injury. This may be aggravated under conditions of high lipid load or redox stress that promotes endothelial cell death. Thus, miR-126-5p expression may serve as a safeguard in case of injury of the endothelial monolayer.

Disclosures
None.

References

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