MicroRNAs
The Swing Voters in Vascular Disease Waiting for a Program

Joost P.G. Sluijter, Gerard Pasterkamp

Atherosclerosis is an inflammatory disease involving, among others, production and degradation of the extracellular matrix and the accumulation of lipids in the arterial wall. Matrix metalloproteinases (MMPs) are the main physiological mediators of matrix degradation, capable of degrading extracellular matrix components such as collagens, proteoglycans, elastin, laminin, fibronectin, and other glycoproteins. The actions of MMPs could potentially be modulated with different approaches: (1) inhibition of MMP expression and protein synthesis, (2) inhibition of MMP activation, and (3) inhibition of MMP activity. Although subject for studies for many years, including the development of more specific inhibitors for different MMP family members such as MMP-8 and MMP-12, opportunities as useful therapeutic targets in clinical studies are still hampered. The effect of broad-spectrum MMP inhibitors has been studied in models of vascular injury in large animal studies with varying outcomes. MMP inhibition results in impaired constricive remodeling after balloon angioplasty but fails to inhibit in stent neointima formation. Interfering with MMP activity has been a popular approach to attenuate atherosclerotic disease in animal models, but translation to human disease is cumbersome. There are many reasons to explain this failure in translation: expression patterns of MMPs and TIMPs (tissue inhibitor of metalloproteinases) in mouse and human macrophages differ, and the lack of adequate preclinical models is evident. In addition, the widespread functional relevance of MMPs in human biology hamper clinical application because of side effects. However, currently clinically applied drugs may have pleiotropic effects that modify the MMP activity and indirectly influence atherosclerotic lesion progression. The search for molecular and genetic mechanisms that modulate MMP activity and thereby affect atherosclerotic disease stabilization remains a challenge.

MicroRNAs in Atherosclerosis and Aneurysm Formation

MicroRNAs (miRNAs) are small regulatory noncoding RNA molecules, 19 to 22 nucleotides in length, which bind to the 3′-untranslated region of the mRNAs and regulate mRNA expression via degradation or inhibition of their translation. miRNAs are thereby able to post-transcriptionally regulate protein levels. Because of the nature of their binding, miRNAs can potentially bind multiple targets, and more and more evidence is linked to a strong regulatory effect of a single miRNA by binding several targets within the same functional network. Evidence is accumulating showing particularly important functional roles for miRNAs in cardiovascular disease, including atherosclerosis and aneurysm formation, which were recently extensively reviewed. miRNAs have been associated with cardiovascular disease risk factors, including hyperlipidemia, hypertension, obesity, diabetes mellitus, lack of physical activity, and smoking. In addition to their cellular role, they have been detected in body fluids as circulating miRNAs, thereby mediating cellular communication, and which makes them potential biomarker candidates.

miRNAs can be functionally blocked by synthetic miRNA inhibitors in vitro and in vivo, which are chemically engineered single-stranded oligonucleotides completely complementary to the 20-nucleotide-long targeted miRNAs. The structure of the inhibitors is modified to improve resistance to nucleases and specificity by increasing their affinity to targeted miRNA.

In the current issue of Circulation Research, Di Gregoli et al report a study on the functional relevance of microRNA-181b (miR-181b) in atherosclerotic lesion development and aneurysm formation. Progressive atherosclerotic plaque development was studied in which macrophages and a balance in matrix turnover, controlled by MMPs and TIMPs, was explored. Granulocyte macrophage colony-stimulating factor, present in unstable atherosclerotic lesions, promotes a more invasive and proapoptotic macrophage phenotype. Di Gregoli et al observed that in macrophages, granulocyte macrophage colony-stimulating factor reduces TIMP-3 protein levels by increasing miR-181b. The authors could demonstrate that miR-181b inhibition promotes a stable plaque and aneurysm phenotype by restoring TIMP-3 expression in macrophages.

Previously, miR-181b has been linked to vascular inflammation in atherosclerosis. Endothelial dysfunction contributes to the development of both acute inflammatory disease states, such as endotoxemia and sepsis, and chronic inflammatory disease states, such as atherosclerosis, diabetes mellitus, rheumatoid arthritis, and inflammatory bowel disease. Interestingly, miR-181b serves as a potent regulator of downstream nuclear...
factor-κB (NF-κB) signaling in the vascular endothelium by targeting IPOA3 (importin-α3), a protein that is required for nuclear translocation of NF-κB.11 By systemic administration of miR-181b mimics, downstream NF-κB signaling was reduced and leukocyte influx in the vascular endothelium decreased, including reduced lung injury and mortality in endotoxemic mice. Systemic delivery of miR-181b, thereby increasing miR-181b levels, protects ApoE−/− mice from atherosclerosis, by reducing the expression of IPOA3 and NF-κB p65 nuclear translocation in the vascular endothelium of lesions.12 Interestingly, enhancing miR-181b levels in macrophages could lead to a more M2 phenotype in atherosclerotic lesions, by targeting Notch1, thereby reducing total plaque areas, necrotic lesions, and cellular infiltration.13 Although these data seem counterintuitive to the study of Di Gregoli et al, where miR-181b inhibition could prevent atherosclerotic lesion progression, the delivery strategy of precursor miRNAs is still not as good developed as the delivery of miRNA inhibitors. Here, miR-181b was intravenously injected on binding to lipophilic carriers, in a concentration that would probably only target circulating cells and the endothelial layer. Hereby, one could specifically target the endothelial barrier and not the leucocytes, whereas in the study by Di Gregoli et al, potent miRNA inhibitors are used in a much higher concentration and thereby potentially affects multiple cell types. These examples, also highlighted in Figure, demonstrate the need for better and more specific targeting of miRNA therapeutics,14 nicely demonstrated by miR-181b being expressed in multiple cell types and thereby regulating different targets. Although potential side effects might be reduced because a specific miRNA can have different targets in different cell types, or for example, different isoforms are used for importin-α isoforms in endothelial cells (IPOA3) and peripheral blood mononuclear cells. Therefore, miR-181b-mediated effects occurred primarily in the vascular endothelium and independent of NF-κB inhibition in lesional macrophages or peripheral blood mononuclear cells.13

**Future Perspective**

miR-181b is a nice example of newly discovered regulatory noncoding RNA molecules that have a powerful regulatory effect. The strong phenotypic effects in the preclinical models suggest that introducing both precursor miRNAs as well as miRNA inhibitors might be worth for future interventions to prevent unstable plaque formation and destabilization of aortic walls and thereby reducing vascular rupture. However, although promising as new therapeutic strategies, one have to take into account that still more and better targeting, for example, as demonstrated via E-selectin–targeted delivery of microparticles that carry miR-181b,16 is needed to specifically target most successful cell types for stabilization.

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**Figure. Proposed roles of microRNA-181b (miR-181b) in atherosclerosis.** GM-CSF indicates granulocyte macrophage colony-stimulating factor; MMP, matrix metalloproteinase; and NF-κB, nuclear factor-κB. Figure adapted from Servier Medical Art, via a Creative Commons Attribution 3.0 Unported License, with data derived from *An et al,13 Di Gregoli et al,9 Sun et al,12 and Li et al.15*
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None.

References

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