The ability of the endothelium to produce nitric oxide (NO) is important for the maintenance of vascular homeostasis, and either the altered expression of the endothelial NO synthase (eNOS) or its activity is thought to make a major contribution to the pathogenesis of vascular disease. The expression of eNOS is tightly regulated and numerous physiological and pathophysiological stimuli have been identified that modulate eNOS gene transcription, mRNA processing, and mRNA stability. A role for microRNAs (miRNAs) in the regulation of eNOS has long been suspected because knocking down Dicer, which is necessary for miRNA maturation, increased eNOS expression.1 In addition, miR-221 and miR-222 mimics partially reversed the increase in eNOS elicited by Dicer down-regulation, although neither of these miRNAs directly targets the eNOS mRNA.1 In this issue of Hypertension, Sun et al2 report that miR-155 directly targets eNOS mRNA by binding to its 3’ untranslated region (UTR) to decrease enzyme expression and NO production.

Which physiological situation could involve the regulation of eNOS expression via miR-155? There are several stimuli that have been reported to decrease eNOS levels and these are generally linked with inflammation, for example, proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin-1, interferon-γ, and bacterial lipopolysaccharide. Using these stimuli, Sun et al2 were able to demonstrate that proinflammatory cytokines increase miR-155 expression, most probably via AP-1 and nuclear factor-κB, and that inhibiting miR-155 partially prevents the TNF-α–induced decrease in eNOS expression in human endothelial cells. These findings could be confirmed in rings of isolated human internal mammary arteries inasmuch as the adenosinergic overexpression of miR-155 decreased both eNOS expression and acetylcholine-induced endothelium-dependent relaxation. Again, the inhibition of miR-155 attenuated the TNF-α–induced decrease in eNOS levels as well as the blunted response to acetylcholine observed in cytokine-treated artery rings.

miRNAs can affect protein expression by binding to and promoting the degradation of target miRNAs or by preventing their translation. In the case of miR-155 and eNOS, the former mechanism seems to predominate as the overexpression of miR-155 significantly shortened the half-life of eNOS mRNA. This is worth mentioning because even before miRNAs were widely recognized as posttranscriptional regulators of expression, TNF-α had already been reported to destabilize eNOS mRNA by a mechanism involving the binding of a number of 50- to 70-kDa cytosolic proteins to specific regions within its 3’-UTR.3,4 miRNA-155 binds to its so-called seed sequence (7 base pairs of complementarity) within the eNOS 3’-UTR, and Sun et al2 were able to show that mutating the miRNA-binding sequence prevented the miR-155–induced downregulation of eNOS protein in a concentration-dependent manner. Whether this seed sequence is in the vicinity of the mRNA segments to which the cytosolic proteins bind and whether these unidentified proteins are part of the RNA-induced silencing complex is unknown.

**Figure.** Tumor necrosis factor (TNF)-α–induced, miR-155–mediated, downregulation of endothelial NO synthase (eNOS). Inflammatory factors, such as TNF-α, increase miR-155 via the activation of nuclear factor (NF)-κB, activator protein (AP)-1, and Rho kinase. MiR-155, together with as yet unidentified cytosolic RNA-binding proteins (RNPs), then bind to and destabilize eNOS mRNA, resulting in a decrease in eNOS protein and NO production. Statins, miR-155 inhibition (anti-miR-155), and Rho kinase inhibitors prevent the increase in miR-155 and thus maintain eNOS levels and NO production.
Over the years, there has been a lot of interest in the effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) on eNOS expression and activity. To date, several mechanisms have been reported to account for the statin-induced upregulation of the enzyme including a not entirely defined mechanism involving eNOS mRNA, geranylgeranylated RhoA, and actin. The study by Sun et al. adds another piece to the puzzle as simvastatin effectively prevented the TNF-α-induced decrease in eNOS expression at the same time as decreasing miR-155 levels. The authors were also able to link the latter effect to RhoA as 2 RhoA inhibitors (C3 exoenzyme and Y-27632) attenuated the TNF-induced upregulation of miR-155 and downregulation of eNOS.

miR-155 regulates several pathways involved in cell division and immunoregulation. It is highly expressed in endothelial cells as well as dendritic cells and has largely been characterized as a proinflammatory miRNA because its upregulation also leads to an increase in TNF-α translation. Moreover, miR-155 governs the ability of dendritic cells to efficiently interact with T cells, to induce their proliferation, and repolarizes tumor-associated macrophages to proinflammatory M1 macrophages. The angiotensin II type 1 receptor (AT1R) is also a target of miR-155 in different cells, and miR-155 translationally represses the expression of AT1R in vivo (for review, see Busch and Zernecke). Thus, an increase in miR-155 would be expected to decrease AT1R levels at the same time as decreasing eNOS.

Perhaps one of the most impressive aspects of many of the miRNAs linked to pathophysiology has been the high rate of translation between the murine and human systems and vice versa. Thus, the logical next step for Sun and colleagues would be to assess the consequences of miR-155 inhibition in a murine model of vascular disease. Somewhat surprisingly, Sun et al. found that miR-155 inhibition was unable to elicit the downregulation of eNOS in mouse aortic endothelial cells even though the murine eNOS mRNA 3′-UTR does contain a putative binding site for miR-155 (albeit with 6 instead of 7 base pairs of complementarity) and miR-155 has been successfully targeted by others in mice.

The miR-155 7-mer seed sequence in the eNOS mRNA 3′-UTR is however highly conserved in primates so what about the clinical situation? It has been reported that miR-155 levels in blood are reduced in patients with coronary heart disease, a finding paralleled by a marked decrease in the expression of miR-155 in the peripheral blood mononuclear cells of patients with acute coronary syndrome. In human atherosclerotic plaques and in macrophages treated with oxidized low-density lipoprotein, on the other hand, miR-155 levels are reportedly increased. In this context, the link between miR-155 and the AT1R is particularly interesting because the gene is highly polymorphic and the AT1R 1166A/C polymorphism has been found to disrupt the miR-155 seed sequence and attenuate its binding to the AT1R 3′-UTR, which may go some way to accounting for the increased frequency of cardiovascular disease associated with this particular polymorphism. There are of course other conditions in which miR-155 levels are reportedly altered including several cancers (miR-155 is a so-called oncogenic miRNA) and autoimmune conditions, including rheumatoid arthritis, which have also been associated with the accelerated development of vascular disease. Given that miR-155 has a powerful regulatory potential in a wide variety of immune cells, and affects vascular homeostasis by affecting responsiveness to angiotensin II as well as NO production, miR-155 is a potential versatile therapeutic target for inflammatory disease.

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References
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Lei Shi and Ingrid Fleming

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