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transcriptional pathway and their induction acts to limit the intensity and the duration of the inflammatory response. These include proteins that antagonize the NF-κB signaling pathway, such as IκBα and A20, as well as several microRNAs, most prominently, miR-146a. MicroRNAs bind primarily to the 3′ untranslated regions of target mRNAs and suppress protein production. In the case of miR-146a, multiple components of proinflammatory signaling pathways (including TRAF6, IRAK1, IRAK2, MyD88, RelB, STAT1, CARD10, and TLR4) have been identified as bona fide targets. Deletion of miR-146a in mice has revealed that this microRNA dampens and extinguishes inflammatory signaling cascades. Mice deficient in miR-146a have prolonged T-cell responses, excessive and lethal responses to endotoxin and develop autoimmune and tumors. These phenotypes are invariably associated with enhanced and prolonged NF-κB–dependent responses.

Cellular levels of miR-146a may affect the susceptibility to inflammation-related diseases. Intriguingly, a single-nucleotide polymorphism in the transcript that is processed into mature miR-146a affects the amount of miR-146a made and is associated with risk of coronary artery disease in certain populations. Although miR-146a is assumed to be protective against atherosclerosis because of its ability to suppress NF-κB signaling and endothelial cell activation, levels of miR-146a are paradoxically elevated in human atherosclerotic plaques, as well as in the circulation and plaques of atherosclerotic mice (ie, Apoe−/− mice). This may be because of the strong activation of NF-κB signaling in plaque, implying that miR-146a is induced as part of a negative feedback loop. Understanding the mechanisms that control the cellular levels of miR-146a will yield insight into pathways that might be involved in regulating atherogenesis. Although transcriptional mechanisms are undoubtedly involved, recent studies have shown that miR-146a is present in the circulation and transfer to recipient cells may also modulate the steady state levels of this microRNA. In addition, it is noteworthy that a network of microRNAs have been uncovered that impinge on inflammatory signaling pathways, and this includes miR-21 and miR-147 (identified as apoE-regulated microRNAs by Li et al). It is therefore likely that miR-146a is not the only microRNA that affects vascular inflammation. Indeed, mouse models of atherosclerosis have revealed an antiatherogenic role for miR-181b, which suppresses the nuclear import of NF-κB proteins in the endothelium, and a proatherogenic role for miR-155, which represses Bcl6, a negative regulator of NF-κB in macrophages.

Li et al now provide compelling evidence that apoE, which is highly expressed by monocytes and macrophages, can positively regulate miR-146a transcription and expression in these cells, and thereby suppress NF-κB activation and inflammatory gene expression (see Figure). The extent to which augmented miR-146a expression contributes to the constellation of beneficial, lipid-independent effects of apoE remains to be fully elucidated, and it would be informative to assess the anti-inflammatory effect of apoE on miR-146a null monocytes/macrophages. It is possible that the regulation of additional microRNAs (such as miR-21 and miR-147a) or NF-κB–independent pathways may contribute to apoE’s effects.

Figure. The role of apoE in regulating proinflammatory gene expression—what is known and what remains to be discovered. ApoE can induce the expression of the transcription factor, PU.1. However, the relevant signaling pathways and receptor utilization have not been defined. PU.1 regulates the transcription of miR-146a and potentially additional miRNAs and microRNAs. MiR-146a suppresses the expression of adaptor proteins that associate with interleukin-1 receptor (IL-1R) and Toll-like receptors (TLRs) and thereby controls nuclear factor-κB (NF-κB) signaling and the induction of proinflammatory genes. Other pathways, such as tumor necrosis factor receptors (TNFR) are not affected by miR-146a. Whether miR-146a also regulates additional proinflammatory signaling pathways or antiapoptotic gene remains to be investigated. Intravascular injection of miR-146a mimetic can suppress atherosogenesis in mouse models. This may be mediated by delivery to monocytes/macrophages and additionally may involve delivery to endothelial cells to suppress their activation, which is required for monocyte recruitment to plaque. Known pathways or interactions are shown as dashed lines, whereas undefined pathways are shown as solid lines. LDL indicates low-density lipoprotein.

The authors show that apoE induces the expression of PU.1, a transcription factor that has been previously implicated in miR-146a transcription. Exploring whether additional PU.1 target genes (including microRNAs and mRNAs) contribute to the apoE anti-inflammatory phenotype seems warranted. A major unanswered question is how apoE induces PU.1. Is this a receptor-dependent or -independent effect, and what signaling pathway(s) are involved?

An important and exciting finding in the article is that intravascular injection of a miR-146a mimetic can recapitulate the protective effects of apoE in both Ldlr−/− and Apoe−/−;Ldlr−/− mouse models of atherosclerosis. This represents a novel potential therapeutic strategy. However, the mechanisms by which the miR-146a mimetic achieves atherosclerosis protection remain to be fully explored. Is inhibition of NF-κB signaling the only mechanism? Is the mimetic primarily delivered to myeloid cells? Presumably elevated miR-146a in peritoneal macrophages reflects that the mimetic was also delivered efficiently to macrophages in atherosclerotic lesions. Of note, the same intravascular injection technique was used.
to efficiently deliver a miR-181b mimic to arterial endothelium, where this microRNA suppressed NF-kB activity through antagonism of nuclear import of NF-kB subunits. Thus, it will be important to determine whether miR-146a mimic is also delivered to vascular endothelium, where miR-146a is capable of efficiently suppressing endothelial cell activation. This is an important consideration in light of previous atherosclerosis studies in which NF-kB signaling was inhibited specifically in either endothelial cells or macrophages. These studies showed that inhibition of NF-kB activation in endothelial cells reduced atherogenesis, whereas inhibition of NF-kB activation in macrophages resulted in enhanced atherosclerosis in Ldlr−/− mice.

Atherosclerosis is a chronic and complex disease that progresses through distinct stages. Macrophage accumulation in early lesions is dependent on endothelial cell activation and monocyte recruitment. Later stages are characterized by stable or diminishing macrophage burden, macrophage renewal dependent on proliferation rather than monocyte recruitment, increasing myeloid cell apoptosis, secondary necrosis because of defective efferocytosis and formation of a necrotic core. Inhibition of inflammation in the early stages of atherosclerosis reduces the extent of lesions, delays the transition to subsequent stages, and influences the progression and characteristics of mature lesions that include smooth muscle cell infiltration, matrix deposition, fibrous cap formation, and calcification. NF-kB activation in endothelial cells induces the expression of leukocyte adhesion molecules and chemokines required for monocyte recruitment, and genetic deletion of endothelial NF-kB signaling or of the resultant proinflammatory genes results in reduced atherosclerotic lesion formation. However, NF-kB also mediates increased expression of genes that protect cells from apoptosis and potentially contribute to the resolution of inflammation. Inhibition of NF-kB signaling in macrophages may induce increased apoptosis and necrosis in advanced lesions and may decrease the production of interleukin-10, an anti-inflammatory cytokine. Thus, it seems that the role of NF-kB signal transduction in atherosclerosis is complex, and therapeutically targeting a specific cell type at a particular stage of atherogenesis will likely be important. Perhaps through fine-tuning of NF-kB signaling, miR-146a can attenuate macrophage inflammatory gene expression without eliciting detrimental effects on macrophage survival.

MicroRNAs represent an exciting therapeutic target for several human diseases, and preclinical studies have shown utility in modulating atherogenesis through manipulation of microRNA-based pathways. The first human trials of microRNA-based therapeutics are underway and there is reason for optimism. For instance, a promising Phase II clinical trial has revealed that miraviren, a miR-122 inhibitor, can inhibit hepatitis C virus replication. In addition, a first-in-class Phase I clinical trial is assessing liposome-mediated microRNA mimic delivery to replace a tumor suppressive microRNA, miR-34, in liver cancers and hematologic malignancies. There are several significant challenges that must be overcome for successful application of microRNA mimetic approaches in the clinic, especially in atherosclerosis, a disease that develops over decades. Defining a prolonged treatment regimen may be difficult, impractical, and costly, and intravascular delivery will be problematic in terms of compliance. Technological advances, such as microRNA mimetic-eluting stents, may be required to make this type of therapeutic approach practical. Delivery to the desired cell type to limit off-target effects is a major hurdle, especially with intravascular administration of liposomes, where numerous cell types are likely targeted. Because NF-kB in macrophages plays a key role in mediating host defense, it will be vital to ensure that delivery of miR-146a mimetics (or other microRNAs that inhibit NF-kB activation) to macrophages will not adversely affect responsiveness to pathogens. Perhaps therapeutically enhancing the regression of established plaques may be more practical than attempting to inhibit plaque formation. One can begin by investigating the role of miR-146a and other microRNAs in atherosclerosis regression models. The findings by Li et al have certainly provided further motivation to pursue miR-146a manipulation in atherosclerosis and other inflammatory diseases.

**Sources of Funding**

J.E. Fish is supported by research grants from the Canadian Institutes of Health Research (CIHR: MOP-119506 and MTA-118968), the CIHR Vascular Network, and the Canadian Cancer Society (Innovation Grant, 702835), the Canada Research Chairs Program and an Early Researcher Award from the Heart and Stroke Foundation of Ontario. M.I. Cybulsky is supported by research grants from CIHR (MOP-84446, MOP-106522, and MOP-89740), Canada Research Chairs Program, and Career Investigator Awards (2002–2014) from the Heart and Stroke Foundation of Ontario.

**Disclosures**

None.

**References**


**Keywords:** apolipoproteins ■ atherosclerosis ■ inflammation ■ microRNAs ■ signal transduction
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Circ Res. 2015;117:3-6
doi: 10.1161/CIRCRESAHA.115.306733

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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