Boosting Endothelial Autophagy by MicroRNA Delivery Quenches Vascular Inflammation

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Autophagy in endothelial cells is an emerging anti-inflammatory and atheroprotective mechanism. In this issue of *Circulation Research*, Pankratz et al[1] describe the function of an endothelial-enriched microRNA, miR-100, that promotes autophagy through direct suppression of the autophagy inhibitors, mTOR (mammalian target of rapamycin) and Raptor. Furthermore, they demonstrate that miR-100 suppresses proinflammatory NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling and endothelial cell activation in an autophagy-dependent manner. Global inhibition of miR-100 in an in vivo model of atherosclerosis worsens disease while the opposite is the case when miR-100 mimetics are delivered intravenously. This study implies that therapeutics that can augment miR-100 levels in the endothelium will enhance autophagy and may be used to restrain vascular inflammation and atherogenesis. However, improvements in the specificity of microRNA delivery in vivo will be required to further test this concept because the authors also identify additional effects on cholesterol metabolism (albeit beneficial) in the livers of miR-100 mimetic-injected mice that seem to be autophagy dependent.

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The activation of the endothelium and subsequent recruitment of circulating inflammatory cells into the vessel wall is a central driver of atherosclerotic disease. This chronic vascular inflammatory response is primarily orchestrated by the transcriptional induction of a network of adhesion molecules, cytokines, and chemokines through the activity of the transcription factor, NF-κB, which is activated downstream of cholesterol accumulation in the vessel wall, and in response to disturbed blood flow patterns. Identifying the mechanisms that control the activation of NF-κB may reveal new therapeutic targets to block disease progression. However, it is important to note that targeting this pathway may only be effective in particular cell types or at specific stages of disease. For example, although endothelial NF-κB is required for atherosclerotic progression,[2] suppression of macrophage NF-κB paradoxically accelerates atherosclerosis.[3]

One NF-κB regulatory control point that has received a great deal of attention is the microRNA pathway. MicroRNAs post-transcriptionally regulate target genes by binding to the 3′ untranslated region of mRNAs and suppressing their stability and translation. A large number of microRNAs have been identified that suppress NF-κB activity in endothelial cells (ECs), either by targeting mRNAs that encode components of upstream signaling pathways, NF-κB regulators or the machinery that mediates NF-κB nuclear import.[4] Recently, autophagy has also been linked to the regulation of NF-κB signaling components in several cell types.[5] Furthermore, several microRNAs target constituents of the autophagy pathway to either enhance or repress this process.[6]

Autophagy is a catabolic process that is essential for the maintenance of homeostasis in the cardiovascular system. Although this pathway facilitates the lysosomal breakdown of organelles and protein aggregates to supply biofuels under conditions of cellular stress, it is also an important quality control mechanism that can remove damaged organelles, including reactive oxygen species–producing mitochondria. In general, autophagy has been shown to have a protective role in ECs, vascular smooth muscle cells, and macrophages in the setting of atherosclerosis; and age-related declines in the efficiency of autophagy and a corresponding increase in damaged organelles have been observed in the aging cardiovascular system.[7] Interestingly, robust autophagic flux occurs in the endothelium in vascular regions that are exposed to high levels of laminar shear stress and are thereby protected from atherosclerosis.[8] In contrast, autophagy is deficient in atheroprone regions that experience disturbed flow. Autophagy not only represses endothelial activation in regions of high laminar flow but is also involved in the alignment of cells in the direction of flow. Endothelial-specific deletion of an essential autophagy gene (*Atg5*) in hypercholesterolemic mice enhances atherosclerosis exclusively in regions of high laminar shear stress.[8] Conversely, enhancing autophagy in ECs exposed to disturbed flow enhances the flow-dependent alignment of these cells and suppresses their inflammatory activation. In addition, endothelial autophagy has been shown to suppress lipid retention in the vessel wall.[9] Strategies that augment autophagy in the endothelium are, therefore, expected to suppress atherogenesis. Although this could be achieved by available inhibitors of mTOR activity, such as rapamycin, global inhibitors may also have unintended effects, such as immunosuppression and hyperglycemia.[10]

In this issue of *Circulation Research*, Pankratz et al[1] demonstrate that miR-100 attenuates inflammation and atherosclerosis by stimulating endothelial autophagy (summarized in Figure). Previously, this research group identified a role

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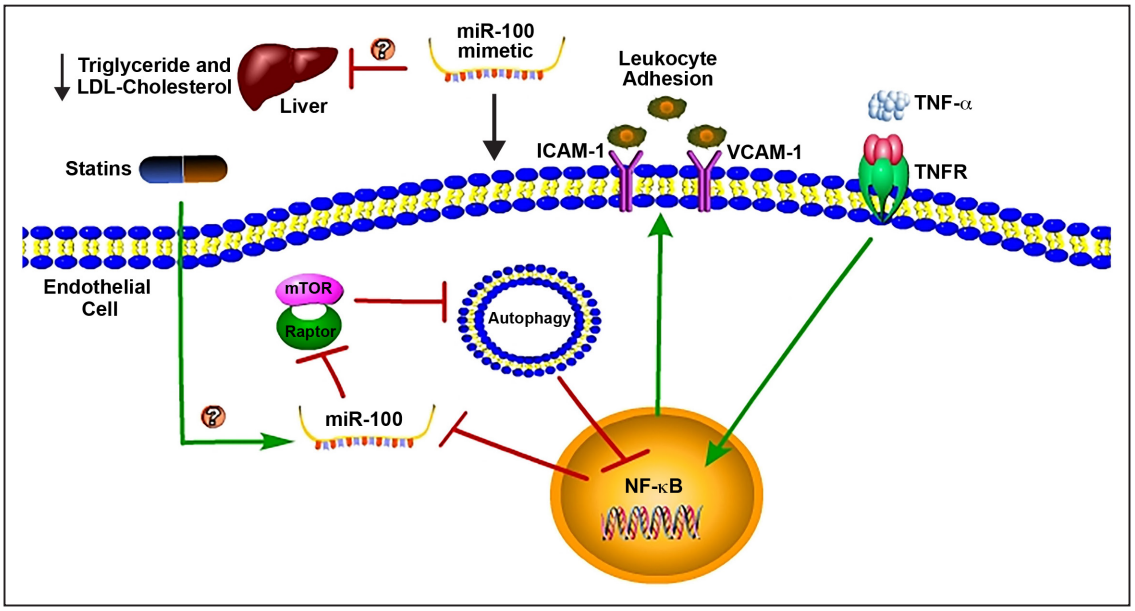

Figure. miR-100 suppresses inflammation in endothelial cells and decreases triglyceride and low-density lipoprotein (LDL) cholesterol levels by activating autophagy. ICAM-1 indicates intercellular adhesion molecule-1; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α, tumor necrosis factor-alpha; TNFR, tumor necrosis factor receptor; and VCAM-1, vascular cell adhesion molecule-1.

for miR-100 in the suppression of angiogenesis through the targeting of mTOR in ECs.[11] Now they report that an additional mTORC1 (mTOR complex 1) component, Raptor, is also a target in the endothelium.[1] Overexpression of miR-100 or inhibition of mTOR activity with rapamycin enhanced autophagic flux, attenuated NF-κB activity, and repressed adhesion molecule levels in ECs, resulting in reduced leukocyte recruitment. The authors also showed that exposure to the proinflammatory cytokine, TNF-α (tumor necrosis factor-alpha), led to a reduction in miR-100 expression in an NF-κB–dependent manner. In contrast, treatment with a statin (ie, simvastatin) enhanced miR-100 expression in vitro and in vivo. The authors elegantly demonstrated that miR-100 antagomirs enhanced leukocyte adhesion and NF-κB activation in an in vivo mouse model. Furthermore, global inhibition of miR-100 exacerbated atherogenesis in *Ldlr*$^{-/-}$ (low-density lipoprotein receptor deficient) mice, whereas upregulation of miR-100 through intravenous injection of miR-100 mimetics decreased lesion area and macrophage content. Importantly, the authors were able to highlight the potential clinical relevance of their preclinical model by observing that miR-100 levels were lower in vulnerable human plaques characterized by fatty lesions with high macrophage content and elevated levels of inflammatory markers.

The ability of miR-100 mimetic injections to reduce macrophage accumulation in atherosclerotic plaques provides an impetus to further develop this approach as an anti-inflammatory therapy and is suggestive of an important role of this microRNA in the regulation of endothelial activation. However, the cell types that took up the injected mimetic in vivo were not precisely mapped, and it is likely that delivery to multiple cell types may contribute to the antiatherosclerotic phenotype. Overexpression of miR-100 in monocytes had a modest effect on their migration, CD11b levels, mTOR expression, and the production of inflammatory cytokines. Thus, delivery of miR-100 mimetic to these cells in vivo may only subtly contribute to disease attenuation. However, there were robust effects on cholesterol metabolism in the liver, with decreased levels of circulating triglycerides and low-density lipoprotein cholesterol. This was accompanied by suppression of the miR-100 target, mTOR, in the liver. The relative contribution of endothelial versus hepatic miR-100 delivery and the extent to which autophagy-independent pathways are affected by miR-100, remains to be determined. Because miR-100 also represses neovascularization,[11] it will also be important to determine whether this might be detrimental in the setting of wound repair, such as after myocardial infarction.

The prospect of delivering miR-100 mimetics to treat vascular inflammatory diseases is enticing. However, there are significant hurdles to the implementation of this type of therapy. Specific delivery to cells of interest remains a major goal of such approaches because this should reduce potential toxicity and off-target effects of global microRNA overexpression. Recently, Dahlman et al[12] developed polymeric nanoparticles that were preferentially taken up by ECs, which facilitated the efficient vascular delivery of short interfering RNAs without affecting target gene expression in hepatocytes or immune cells. Developing such tunable nano-formulations that are translatable to microRNA mimetics has the potential to safely extend the clinical use of miR-100 mimetics and could also be used to directly test miR-100 delivery to the endothelium, without confounding effects in the liver. An additional challenge is achieving sustained microRNA overexpression, especially in the setting of a chronic disease, such as atherosclerosis. Development of microRNA therapeutics remains an active and exciting area of clinical development. For example, miR-122 antagomirs have been shown to be effective in controlling viral load in a phase 2a study of patients with hepatitis

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C.[13] However, microRNA replacement therapies have had a bumpier road to the clinic, with a recent phase 1 clinical trial in patients with cancer being halted after several reports of cytokine release syndrome in patients.[14] Further advances are clearly needed in the arena of microRNA-based therapies.

Altogether, this exciting study by Pankratz et al[1] expands our armamentarium against inflammation and atherosclerosis by uncovering a new anti-inflammatory microRNA that acts by enhancing autophagic flux in the endothelium.

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Disclosures

None.

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