The accumulation of cholesterol in the arterial wall initiates the progression of atherosclerosis, which is one of the major causes of death in Western societies. Excess cholesterol must be removed and transported from the peripheral tissues to the liver for its reutilization or its excretion into feces in a physiological process traditionally known as reverse cholesterol transport. During reverse cholesterol transport, plasma high-density lipoprotein (HDL) is thought to function as a sterol transporter that facilitates the movement of sterols from peripheral cells to the liver. In addition to its role in regulating reverse cholesterol transport, many studies have shown that HDL may also have antiatherogenic properties. Indeed, HDL decreases endothelium inflammation and oxidative stress and increases nitric oxide production and endothelial cell (EC) survival, thus preventing atherogenesis. Although these observations have been reported in several studies, the molecular mechanisms underlying these effects are still unclear.

In a recent report published in the February 28, 2014, issue of *Nature Communications*, Tabet et al showed that HDL can transfer microRNAs to ECs, influencing gene expression in the recipient cell. MicroRNAs are small noncoding RNAs that regulate gene expression at the post-transcriptional level by inhibiting the translation or decreasing the stability of mRNA target genes. The authors found that ECs treated with native HDL (nHDL) showed increased levels of microRNA-223. This microRNA was reducing EC inflammation by directly targeting intercellular adhesion molecule 1 (ICAM-1). The enrichment of microRNA-223 in ECs was mediated by HDL cargo delivery to the recipient cells because their incubation with other HDL components, such as apolipoprotein A-I or recombinant HDL, did not influence the endothelial levels of microRNA-223. The authors used many elegant experimental approaches to demonstrate that the microRNA transfer occurs between nHDL and ECs in vitro. For instance, to avoid the confounding effect of endogenous microRNA-223 in ECs, the authors treated ECs with actinomycin D (to inhibit de novo transcription) or silenced Dicer expression using a small interfering RNA (to inhibit endogenous microRNA-223 maturation) in the presence of nHDL. In both experiments, microRNA-223 levels remained similar to untreated controls (absence of actinomycin D or scrambled siRNA), demonstrating that nHDL efficiently transfers microRNA-223 to ECs.

To assess the functional relevance of microRNA-223 in ECs, the authors analyzed microRNA-predicted targets using bioinformatic algorithms (TargetScan). Interestingly, they found ICAM-1, a glycoprotein that regulates vascular inflammation by facilitating leukocyte recruitment, and colony-stimulating factor 2, a cytokine that controls the production, differentiation, and function of macrophages, as predicted microRNA-223 target genes. To demonstrate that microRNA-223 regulates ICAM-1 and colony-stimulating factor 2 expression at the post-transcriptional level, the authors cloned the 3′ untranslated region of both genes in a luciferase reporter vector and assessed luciferase activity after overexpressing microRNA-223. The results indicated that microRNA-223 downregulated ICAM-1 and colony-stimulating factor 2 expression levels. More interestingly, microRNA-223 diminished ICAM-1 protein expression in proinflammatory conditions (ECs treated with proatherogenic cytokines, such as tumor necrosis factor-α).

Finally, the authors tested the role of HDL-derived microRNA-223 in regulating EC activation by comparing the anti-inflammatory effect of HDL isolated from wild-type and microRNA-223-deficient mice. Notably, ECs treated with HDL isolated from wild-type mice diminished ICAM-1 and colony-stimulating factor 2 levels. However, this anti-inflammatory effect was lost in ECs treated with HDL isolated from microRNA-223-deficient mice, suggesting that HDL-derived microRNA-223 plays an important role in the well-described anti-inflammatory properties of HDL.

One important question that needs to be addressed is the mechanism by which the microRNAs are transferred between HDL and ECs. Previous work from the Ramaley Laboratory demonstrated that the scavenger receptor B1 was critical for the uptake of microRNAs in human hepatic cell lines (Huh7). Because scavenger receptor B1 is also expressed in ECs, it could be possible that the same receptor may mediate the HDL-derived microRNA transfer to ECs.

Other groups have also studied the potential transfer of HDL-containing microRNAs to ECs. Dimmeler and colleagues found that microRNA-223 was the most abundant microRNA in HDL, but they were unable to demonstrate the transfer of microRNAs between HDL and ECs. Moreover, they did not find differences in the microRNA content of HDL isolated from healthy control subjects and patients with stable coronary artery disease or acute coronary syndrome. The discrepancies between the results obtained by both groups...
might be explained by the different origin of ECs used in their respective studies. Although Tabet et al. used primary human coronary aortic endothelial cells, Wagner et al. performed their studies in human umbilical venous endothelial cells. The different expression levels of scavenger receptor B1, as well as other receptors that mediate microRNA transfer between HDL and ECs, in human coronary aortic endothelial cells and human umbilical venous endothelial cells might address this discrepancy. It is also important to note that the study of cellular transport in ECs in vitro is challenging for several reasons, including the loss of endothelial glycocalyx that controls lipoprotein retention and mechanotransduction; the absence of caveolae observed in primary ECs cultured in vitro; and the loss of EC polarization that may influence membrane receptor localization. Therefore, to definitely demonstrate the biological significance of these findings, the transfer of HDL-derived microRNAs should be tested using an in vivo model or in canulated vessels.

In summary, this interesting study shows the potential transfer of HDL-associated microRNAs to ECs and provides a novel mechanism by which HDL might regulate EC activation. Additional studies of how HDL-derived microRNAs might influence gene expression in other cells associated with atherosclerotic vascular disease, such as macrophages and vascular smooth muscle cells, might be of interest.

**Sources of Funding**

Research in the Fernández-Hernando laboratory is supported by funding from the National Institutes of Health (R01HL107953 and R01HL106063).

**Disclosures**

None.

**References**


**Key Words:** commentary  ■  endothelium  ■  HDL  ■  microRNA  ■  post-transcriptional gene silencing
Antiatherogenic Properties of High-Density Lipoprotein–Enriched MicroRNAs
Carlos Fernández-Hernando

Arterioscler Thromb Vasc Biol. 2014;34:e13-e14; originally published online April 24, 2014;
doi: 10.1161/ATVBAHA.114.303542
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/34/6/e13

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for
which permission is being requested is located, click Request Permissions in the middle column of the Web
page under Services. Further information about this process is available in the Permissions and Rights
Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online
at:
http://atvb.ahajournals.org//subscriptions/