Adeno-Associated Viruses as a Method to Induce Atherosclerosis in Mice and Hamsters

Noam Zelcer, Marit Westerterp

Atherosclerosis involves multiple cell types and organs and can therefore only be recapitulated in animal models. The most widely used mouse models to study atherosclerosis include mice deficient in apolipoprotein E (Apoe<sup>−/−</sup> mice) or the low-density lipoprotein receptor (Ldlr<sup>−/−</sup> mice).<sup>1</sup> Apoe<sup>−/−</sup> mice develop advanced plaques on a chow diet, which is accelerated by Western-type diet (WTD) feeding, whereas Ldlr<sup>−/−</sup> mice require WTD feeding to develop advanced atherosclerosis and show only foam cell–rich plaques on chow.<sup>1</sup> Wild-type mice do not develop atherosclerotic plaques unless fed with the Paigen diet, but this diet is preferably avoided in these studies because it contains cholate that induces steatohepatitis.<sup>1,2</sup>

To study the contribution of genes and pathways to atherogenesis, crossbreeding with the above-mentioned models is required. In this issue of Circulation Research, Bjørklund et al<sup>3</sup> propose an alternative to this procedure and elegantly show that a single injection of recombinant adeno-associated virus 8 (rAAV8) expressing either a mouse or a human gain-of-function mutant for proprotein convertase subtilisin/kexin type 9 (PCSK9) in combination with WTD feeding induces atherosclerosis in mice and hamsters. PCSK9 is a secreted protein that is predominantly produced in the liver and binds an extracellular domain of the LDLR to target it to the lysosome for degradation.<sup>3</sup> Bjørklund et al<sup>3</sup> used rAAV8 expressing mouse D377Y-PCSK9 (rAAV8-D377Y-mPCSK9) or human D374Y-PCSK9 (rAAV8-D374Y-hPCSK9) to induce atherosclerosis in C57BL/6N Tac mice.<sup>3</sup> For both rAAV8s, stable PCSK9 plasma levels were reached 7 to 14 days after injection, leading to >95% reduced hepatic LDLR abundance. As a result, on the WTD and Paigen diet, plasma cholesterol levels were increased in a rAAV8 dose-dependent manner, reaching >50% to 80% of cholesterol levels in Ldlr<sup>−/−</sup> mice on the same diet. On the WTD, the increase in cholesterol was mainly confined to the low-density lipoprotein (LDL) fraction although very low LDL (VLDL) cholesterol also increased at higher rAAV8 doses. At 12 weeks after rAAV8-D377Y-mPCSK9 injection combined with WTD feeding, C57BL/6N Tac mice showed advanced atherosclerotic lesions in the aortic root, which also contained necrotic cores.<sup>3</sup> Atherosclerosis in the aortic arch and branch areas was increased by rAAV8-D377Y-mPCSK9 or rAAV8-D374Y-hPCSK9 in a dose-dependent manner on the WTD, although even at the highest rAAV8 dose, the lesion area was only ≈50% compared with that in WTD-fed Ldlr<sup>−/−</sup> mice.<sup>3</sup> Moreover, rAAV8-D374Y-hPCSK9 increased atherosclerosis in WTD-fed diabetic Akita mice and induced small foam cell–rich lesions in WTD-fed Golden Syrian hamsters, demonstrating the applicability of the AAV-PCSK9 approach for different mouse models and species.<sup>3</sup>

Although this approach clearly forms an advance for studying early atherosclerosis, the suitability of AAV-PCSK9 for studying advanced atherosclerosis may be questioned. In the aortic root, advanced lesions were observed in WTD-fed mice, but lesions in the arch and branch areas were relatively small.<sup>3</sup> These regions are particularly sensitive to shear stress and to the activity of the enzyme endothelial nitric oxide synthase that plays a key role in maintaining endothelial function in the onset of atherogenesis and has a larger effect on atherosclerosis in the aortic arch than in the root.<sup>3</sup> It thus remains to be addressed whether rAAV8-D377Y-mPCSK9 or rAAV8-D374Y-hPCSK9 also induce advanced atherosclerosis in the aortic arch and branch areas after a longer period of PCSK9 expression. Interestingly, studies in transgenic mice expressing hepatic D374Y-hPCSK9 showed that sustained PCSK9 plasma levels in combination with 15 weeks of high-cholesterol diet increased plasma VLDL/LDL cholesterol and atherosclerosis throughout the whole aorta.<sup>6</sup> It is well known that rAAV8 gives long-term expression,<sup>7</sup> suggesting that in the case of rAAV8-D374Y-hPCSK9 also long-term stable plasma levels could be accomplished.

PCSK9 has been reported to preferentially degrade the LDLR in the liver.<sup>8</sup> Nevertheless, a reduction of up to ≈60% of LDLR in adipose tissue, kidney, and lung has been reported in mice injected with a PCSK9-encoding adenovirus.<sup>9</sup> In the rAAV8-D377Y-mPCSK9 model, hepatic LDLR was reduced by >95%, whereas plasma cholesterol levels were ≈50% to 80% of those in Ldlr<sup>−/−</sup> mice.<sup>3</sup> Extrahepatic LDLR in the rAAV8-D377Y-mPCSK9 mice probably contributed to LDL lowering in these mice compared with Ldlr<sup>−/−</sup> mice. Although modest, a contribution of haematopoietic cells to plasma LDL levels has been reported,<sup>10</sup> raising the possibility that other cell types that are insensitive to PCSK9-mediated LDLR degradation contribute to plasma LDL levels. Thus, the rAAV8-D377Y-mPCSK9 model, which effectively creates a liver-specific Ldlr knockout mouse, offers the possibility to investigate the function of nonhepatic LDLR and its contribution to the regulation of plasma LDL levels and atherogenesis.
In addition to PCSK9, another pathway regulating LDLR abundance in the liver has been discovered, involving the inducible degrader of the LDLR (IDOL). IDOL is an E3-ubiquitin ligase that specifically promotes ubiquitination and subsequent lysosomal degradation of the LDLR. IDOL and PCSK9 share an overlapping substrate specificity that includes the LDLR, VLDLr, and APOE receptor 2, yet the transcriptional pathways that govern their expression are divergent. PCSK9 is transcriptionally regulated by the sterol regulatory element–binding protein 2 pathway and is induced together with the LDLR when cellular cholesterol levels decline. In contrast, IDOL is transcriptionally controlled by the transcription factors liver X receptors, and its expression is increased when cellular sterols increase (Figure). Several lines of evidence indicate that while mediating a similar outcome—lysosomal degradation of LDLR—the IDOL and PCSK9 pathways are independent of each other; PCSK9 promotes degradation of LDLR in Idol−/− cells, LDLR internalizes via distinct endocytic routes in response to IDOL or PCSK9, and an LDLR receptor lacking the intracellular tail, which is required for IDOL-dependent degradation, remains sensitive to PCSK9. Short-term adenoviral-mediated expression of mouse IDOL in mouse liver results in a dramatic elevation in circulating levels of LDL cholesterol, which was entirely dependent on Ldlr expression. Therefore, it will be interesting to test whether long-term hepatic expression of IDOL leads to sustained elevated LDL levels and atherogenesis.

The expression of a human transgene to induce atherosclerosis has been reported before in mice transgenic for apolipoprotein B (apoB) or APOE*3-Leiden rats. Several studies of apolipoprotein B transgenic mice show a human-like lipoprotein profile with marked increases in LDL cholesterol, leading to advanced atherosclerosis with larger effects in females than in males; female, but not male APOE*3-Leiden mice show attenuated clearance of LDL/IDL particles and develop advanced atherosclerosis on a WTD. Furthermore, their plasma VLDL/LDL cholesterol level mirrors the dietary cholesterol content, they are responsive to statins, and crossbreeding with mice transgenic for cholesteryl ester transfer protein has extended their application as a model for testing antiatherogenic drugs. The study by Bjørklund et al. shows that injection with rAAV8-D374Y-hPCSK9 may be a highly promising model for atherosclerosis studies. Expression of the same mutant was also shown to increase atherogenesis in Yucatan minipigs. Given that PCSK9 is highly conserved among species, the rAAV8-D374Y-hPCSK9 approach may be generally applicable in several animal models.

**Disclosures**

Dr N. Zelcer is supported by a European Research Council Consolidator Grant 617376. Dr N. Zelcer and Dr M. Westerterp are supported by The Netherlands Organization of Scientific Research (NWO) VIDI grant 17.106.355 and VENI grant 916.11.072, respectively.

**References**


**Figure. Regulation of low-density lipoprotein receptor (LDLR) abundance by proprotein convertase subtilisin/kexin type 9 (PCSK9) and inducible degrader of the LDLR (IDOL) in hepatocytes. Left: When intracellular cholesterol levels decline, transcriptional activity of sterol regulatory element–binding protein 2 (SREBP2) is increased. This induces PCSK9 and LDLR expression. PCSK9 binds LDLR extracellularly and directs it to the lysosome for degradation. Right: When intracellular cholesterol levels increase, transcriptional activity of liver X receptor (LXR) is turned on. This induces IDOL expression. IDOL binds the intracellular tail of the LDLR and promotes ubiquitination (Ub) of the receptor, directing the receptor for lysosomal degradation.**


**Key Words:** Editorials ■ atherosclerosis ■ liver ■ receptors, LDL
Adeno-Associated Viruses as a Method to Induce Atherosclerosis in Mice and Hamsters
Noam Zelcer and Marit Westerterp

_Circ Res._ 2014;114:1672-1674
doi: 10.1161/CIRCRESAHA.114.304067

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
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:
http://circres.ahajournals.org/content/114/11/1672

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ 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 _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/