HHS Public Access

Author manuscript

Curr Opin Lipidol. Author manuscript; available in PMC 2015 April 01.

Published in final edited form as:

Curr Opin Lipidol. 2013 October ; 24(5): 455–456. doi:10.1097/MOL.0b013e3283654ef9.

Atherosclerosis: cell biology and lipoproteins

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Atherosclerosis is a complex disease of the arterial wall in which a multitude of mediators have been implicated in lesion evolution. Although a wide range of factors have been inferred in the development of atherosclerosis, human observations and animal studies have provided strong evidence that hypercholesterolemia plays a major role in development and progression of atherosclerosis. In the past two decades, two hypercholesterolemic mouse models, low-density lipoprotein (LDL) receptor −/− and apolipoprotein (apo) E −/− mice, have been used extensively to study mechanisms of atherosclerosis [1]. To generate genetic mouse models in either of these two backgrounds is both cost and time consuming. This Bimonthly Update summarizes two recent publications [2**,3**] that have introduced a new, rapid approach to inducing hypercholesterolemia in mice [4].

In the early 2000s, the discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9) mutations led to new insights into mechanisms of hypercholesterolemia in humans [5]. It is now well-recognized that a role of this protein is to regulate plasma apolipoprotein B (apoB)-containing lipoprotein concentrations through degrading LDL receptors in the liver [5, 6]. Subsequent studies found that genetic deficiency of PCSK9 in mice led to a significant but minor reduction of plasma cholesterol concentrations compared to their wild type controls (PCSK9 +/+ versus −/−: 96 versus 46 mg/dl) [7]. Conversely, overexpression of human wild-type PCSK9 in C57BL/6 mice increased plasma cholesterol concentrations from 74 to 176 mg/dl [8]. However, plasma cholesterol concentrations in this mouse model [8] were not sufficiently increased to induce atherosclerosis. The effects of manipulating plasma concentrations of PCSK9 on atherosclerosis were drawn attention recently when two independent research groups [2**,3**] reported that a single injection of adeno-associated viruses (AAVs) containing a gain-of-function mutation of PCSK9 provoked profound increases of plasma cholesterol concentrations in mice within days after injections and induced atherosclerosis.

In one study [3**], AAVs containing a gain-of-function mutation (D377Y in mouse or D374Y in human) of PCSK9 was administered through tail vein injection to C57BL/6 male mice, followed by feeding a Western diet or cholate-supplemented Paigen diet for 12 weeks.

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Conflicts of interest

There are no conflicts of interest.

Financial Disclosures

The authors' research work is supported by National Heart, Lung, and Blood Institute of the National Institutes of Health under award number R01 HL107319.

Injections of AAVs containing either PCSK9 mutation led to rapid and profound increases of plasma PCSK9 concentrations in mice fed either diet. These increases were dose-dependent and maintained throughout the study. Mirroring the high plasma concentrations of PCSK9, plasma cholesterol concentrations were increased rapidly within 7 days after injections in a dose-dependent manner and maintained at these concentrations throughout the study. Plasma cholesterol concentrations and profiles of lipoprotein cholesterol in mice injected with a high dose of PCSK9 AAVs were similar to LDL receptor −/− mice fed either diet. As expected in hypercholesterolemic mice, atherosclerotic lesions in aortas were readily apparent in the aorta of these mice. Increases in plasma cholesterol concentrations and atherosclerosis by injections of AAVs containing PCSK9 mutation were also demonstrated in *Akita* diabetic mice and hamsters.

In agreement with studies by Bentzon's group [3**], Roche-Molina and colleagues [2**] also reported that a single injection of AAV containing D374Y mutation of human PCSK9 through the right femoral vein of mice, followed by feeding a saturated fat-enriched diet, resulted in pronounced increases of plasma cholesterol concentrations that were attributed to increases of apoB-containing lipoproteins. As with the study of Bjorklund et al. [3**], PCSK9-induced hypercholesterolemia resulted in the presence of large aortic atherosclerotic lesions in C57BL/6 mice, which were equivalent to lesions in apoE −/− mice fed same saturated fat-enriched diet. In addition to C57BL/6 mice, this study determined effects of PCSK9 mutation on hypercholesterolemia in multiple mouse strains including FVB/NCr1, 129/SvPasCrif, and apoE −/−. Injections of AAVs containing PCSK9 mutation into apoE −/− mice exacerbated hypercholesterolemia and atherosclerosis in apoE −/− mice, which mimicked apoE and LDL receptor double deficient mice, a several hypercholesterolemic mouse model that is difficult to breed [9].

These two studies enable a profound reduction in the time needed to complete atherosclerosis studies, compared to the traditional approach to inducing hypercholesterolemia through manipulation genes by homologous recombination. For example, the determination of effects of a deleted gene on atherosclerosis in LDL receptor −/− mice usually requires over a year to generate the compound deficient mice needed to complete the study. In addition to the expediency of obtaining data on atherosclerosis mechanisms, this approach will enable major cost saving to atherosclerosis studies in mice. The cost of developing AAVs expressing PCSK9 mutants is relatively trivial, especially in relation to the breeding costs associated with development of LDL receptor −/− background with a compound deficiency. It also provides much greater flexibility to generate hypercholesterolemia in different strains of mice for use in gene association studies. It is worth noting that although the current research focuses on PCSK9 degrading LDL receptors, investigators are exploring other functions of this protein [10, 11]. Therefore, both LDL receptor-dependent and independent effects of this protein need to be addressed in future studies.

Acknowledgments

The content in this commentary is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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