Accelerating the Pace of Atherosclerosis Research

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Manipulations of plasma cholesterol concentrations have been the mainstay of experimental atherosclerosis research for many decades. Before the introduction of the widespread use of genetic manipulations, atherosclerosis research primarily relied on dietary manipulations to produce hypercholesterolemic states. This was easily achieved in some species, such as rabbits, by the addition of cholesterol to the diet. Although many animals have been used in the development of atherosclerosis studies, there has been a dramatic increase in focusing on the use of mice to determine mechanisms of the disease. Like many species, mice do not readily respond to elevations of dietary cholesterol to generate a hypercholesterolemic state. The use of mice for atherosclerosis research was pioneered by the landmark studies of Dr Paige1,4 described the appearance and quantification of lesions in the mouse aorta after mice were fed a diet containing high content of cholesterol and cholate.

See accompanying article on page 50

The application of mice to atherosclerosis studies was enhanced dramatically by the manipulation of genes relevant to lipoprotein metabolism. For example, mice deficient in apoE are hypercholesterolemic and develop atherosclerotic lesions even when fed normal laboratory diets.5,6 The extent of hypercholesterolemia and atherosclerosis is enhanced by feeding diets that mimic the fat contents of some fast food companies.7 Another widely used model is the low-density lipoprotein (LDL) receptor knockout mouse, and in this case, the use of a high-cholesterol/high-fat diet is required to achieve hypercholesterolemia and atherosclerosis.8 For both strains of mice, numerous studies have demonstrated that atherosclerosis develops in several vascular areas, particularly in the aortic root and innominate artery, although superficial atherosclerotic lesions can be found in focal areas throughout the aorta.9,10

Since the introduction of these genetically altered animals, there has been an impressive number of potential targets identified that potentially reduce lesion size and their proclivity to rupture.11 Identification of some novel mechanisms in the development of atherosclerosis that led to a proliferation of potential therapeutic targets has been based on the development of mice with compound genetic deficiencies. The availability of these mice has been a great boost to both academic and pharmaceutical industries to develop potential new therapies. However, there are 2 major constraints to the use of compound-deficient mice (ie, time and money). As illustrated in the Figure, the development of genetically deficient animals is a process that commonly takes in a range of 2 years to generate colonies of sufficient sizes for the determination of atherosclerosis. The C57BL/6 genetic background is commonly used, and this mouse strain has a reputation as being poor breeders. Even when breeding schemes are optimized, the development of compound-deficient animals consumes considerable costs, including colony housing personnel to perform management and genotyping.

As a mode of circumventing the need to develop mice in a hypercholesterolemic background to perform atherosclerosis studies, 2 recent studies have demonstrated the ability to promote high plasma cholesterol concentrations using an adeno-associated viral vector (AAV) expression of a mutant form of proprotein convertase subtilisin/kexin type 9 (PCSK9) acutely.12,13 PCSK9 has evoked intense interest in recent years after the discovery that mutations of PCSK9 were the basis for some forms of autosomal dominant hypercholesterolemia.14 PCSK9 regulates plasma cholesterol concentrations through recognition of the extracellular domain of LDL receptors, which then accelerates its intracellular degradation. Several PCSK9 mutants have been identified in humans, including the gain-of-function mutant used in these 2 recent reports.12,13 Both reports12,13 used AAVs as a delivery mechanism to promote chronic expression of gain-of-function mutants that were either human D374Y or mouse D377Y. Both studies demonstrated that the combination of either mutant of PSCK9 expression or feeding fat-enriched diets leads to pronounced hypercholesterolemia and atherosclerosis. The lipoprotein cholesterol distribution in the presence of mutant PCKS9 resembled profiles generated using plasma from LDL receptor−/− mice fed fat-enriched diets. Therefore, both studies demonstrated the ability to develop a phenotype akin to LDL receptor deficiency in mice without the substantial time and effort of breeding mice into genetically deficient in LDL receptors.

Many facilities can develop AAV at reasonable cost, and at the infection rate used in these 2 publications, the cost of the amount of AAV needed to infect mice is minimal. Also, there are no major biosafety concerns using AAVs. Therefore, there can be considerable savings in negating the need to purchase apoE−/− or LDL receptor−/− mice. Because apoE−/− and LDL receptor−/− mice are only available commercially in a C57BL/6 background, this approach will also facilitate the studies that use different strains to search for genes that modify atherosclerotic lesion formation.

Although it is assumed that mice infected with mutant PCSK9 is mimicking responses in LDL receptor−/− mice, the effects of PCSK9 could extend beyond effects on LDL receptors. For example, PCSK9 is also known to interact with other members of the LDL receptor gene family, including VLDL receptor and LRPI (low-density lipoprotein receptor-related

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protein 1). These broader effects have the potential to promote differences between mice expressing mutant PCSK9 and those genetically lacking LDL receptors, which should be investigated extensively in future studies.

The ability to develop hypercholesterolemia in mice represents a major benefit in the use of mouse models to study mechanisms of atherosclerosis acutely. It may also have applicability to other areas. For example, a commonly used model for the development of abdominal aortic aneurysms is infusion of angiotensin II into hypercholesteremic mice. Although angiotensin II–induced abdominal aortic aneurysms can be generated in normolipidemic mice, the incidence is much lower than in hypercholesterolemic mice. As with atherosclerosis studies, the development of compound-deficient animals has been a major impediment to studies. Therefore, validation of the approach of injecting an AAV expressing PCSK9 would be a valuable addition to the literature.

Overall, the recent 2 studies demonstrate that persistent expression of a gain-of-function mutant of PCSK9 leads to chronic hypercholesterolemia in mice and subsequent atherosclerosis. We predict that there will be a rapid assimilation of this approach into atherosclerosis studies, which will greatly accelerate the rate of discoveries on atherosclerosis mechanisms while diminishing costs.

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**References**

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