Study of ABCA1 Function in Transgenic Mice

Charles Joyce, Lita Freeman, H. Bryan Brewer, Jr, Silvia Santamarina-Fojo

Abstract—The ATP-binding cassette transporter A1 (ABCA1), identified in 1999 as the gene defective in Tangier disease, promotes efflux of cellular cholesterol from macrophages and other peripheral tissues to apolipoprotein acceptors. These ABCA1-mediated processes are anticipated to have antiatherogenic properties, prompting the development of pharmacological agents that increase ABCA1 gene expression as well as the establishment of ABCA1-transgenic mouse lines. Preliminary studies of ABCA1-Tg mice seem to validate the selection of this transporter as a therapeutic target for the treatment of low HDL syndromes and cardiovascular disease but have also raised new questions regarding the function of ABCA1. In particular, the relative contribution of hepatic and peripheral ABCA1 to plasma HDL levels and to reverse cholesterol transport, as well as the potential role of ABCA1 in modulating the plasma concentrations of the apolipoprotein B–containing lipoproteins and protecting against atherosclerosis, seem to be promising areas of investigation. The present review summarizes the most recent studies and discusses insights provided by these transgenic mouse models. (Arterioscler Thromb Vasc Biol. 2003;23:1110–1117)

Key Words: high-density lipoproteins  atherosclerosis  cholesterol efflux  aortic atherosclerosis  ABC transporters

In 1999, ABCA1 was identified as the gene defect in Tangier disease,1–7 a condition characterized by decreased plasma HDL cholesterol (HDL-C), sterol accumulation in tissue macrophages, and increased incidence of cardiovascular disease.8,9 Since then, many laboratories have made important contributions to the understanding of ABCA1 function. ABCA1 promotes the first step in reverse cholesterol transport,10,11 namely the efflux of cellular cholesterol and phospholipids to apolipoprotein acceptors.1,6,12–16 The removal of excess cholesterol is of critical importance in preventing cholesterol accumulation and foam cell formation in macrophages.12,17–20 ABCA1 gene expression is under tight transcriptional and posttranscriptional regulation by a variety of different agents in addition to sterols.21–26 Studies in humans and mice with ABCA1 deficiency have provided evidence that ABCA1 function is a major determinant of plasma HDL concentrations and may beneficially influence atherogenic risk. Thus, ABCA1 has emerged as a transporter with major importance in lipoprotein metabolism, macrophage cholesterol homeostasis, and atherosclerosis.14,18–20,27–30 Because of these combined findings, ABCA1 is now considered a major therapeutic target for the treatment of low HDL syndromes and cardiovascular disease.

Recently, several laboratories have generated transgenic mice that overexpress the human ABCA1 gene (ABCA1-Tg). Preliminary analysis of these mice31–33 seems to confirm the anticipated benefits of enhancing ABCA1 expression in vivo. However, these studies have also raised questions regarding the complex role of ABCA1 in modulating plasma lipoprotein metabolism, macrophage cholesterol homeostasis, and atherogenic risk.

ABCA1-Tg Mouse Lines

ABCA1-Tg mice have been created independently in 3 different laboratories to investigate the effects of ABCA1 overexpression on plasma lipids and atherosclerosis.31–33 Although all transgenic mouse lines overexpress human ABCA1, each has its own unique features, including the source and type of the human ABCA1 transgene, the promoter controlling ABCA1 expression (and thus the sites and levels of ABCA1 expression), and the genetic background of the mice. It is therefore of interest to describe the generation of each transgenic mouse strain to interpret the results arising from each transgenic mouse model.

Vaisman et al31 developed two separate lines (hABCA1-A and hABCA1-B) of transgenic mice overexpressing human ABCA1 cDNA4,34 under control of the apolipoprotein E (apoE) promoter35 in a pure C57Bl/6 mouse background. This promoter contains the hepatic control region one (HCR.1) and the multienhancer two (ME.2), which direct hepatic and macrophage-specific gene expression.36,37 The ME.2 region contains a functional LXR element that mediates the regulation of apoE gene expression by oxysterols and retinoic acids.38 In both ABCA1-A Tg and ABCA1-B Tg mice, increased levels of human ABCA1 mRNA and protein were demonstrated in liver (×4 and ×9) and macrophages (×3 and ×6), but overexpression did not occur in brain, adrenals, heart, small intestine, spleen, lung, or kidney, tissues that...
ABCA1 Transgenic Mouse Models

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— indicates no change; NR, not reported; Chow, regular chow diet; CCB, cocoa butter diet.

express endogenous ABCA1.32,33,39,40 These mice were developed to evaluate the effects of ABCA1 on lipid metabolism and atherosclerosis when overexpressed in two key target organs, liver and macrophages.

Cavelier et al.32 created two lines of ABCA1-Tg mice, BAC1 and BAC2, by injecting two human ABCA1-containing BACs with different 5′ ends into mice with a pure FVB background. BAC1 contained a 255-kb ABCA1 insert, which included 70 kb upstream of Exon 1. BAC2 contained a 171-kb ABCA1 insert beginning in intron 1, 13 kb upstream of Exon 2. Despite the absence of Exon 1 and its promoter, BAC2 mice were able to initiate transcription of the ABCA1 gene because of a newly characterized intron 1 promoter, leading to a new exon termed Exon 1A (+1 for Exon 1A is 2210 bp upstream of Exon 2). Both transcripts were shown to be naturally present in human tissues, with the Exon 1A transcript most abundant in human liver. The BAC1 mice had expression of human ABCA1 equivalent to endogenous mouse ABCA1 in liver, spleen, lungs, and heart and somewhat higher expression levels in macrophages but lower levels of overexpression in brain, intestine, kidney, and adrenals compared with the endogenous mouse ABCA1 levels.32,41 The BAC2 mice expressed the human transgene almost exclusively in testis and liver, with very highly increased levels of the human ABCA1 mRNA in testis, >2-fold increased levels in liver, and trace amounts in intestine and spleen compared with the endogenous mouse transcript. These studies were the first to demonstrate the presence of a previously unrecognized ABCA1 gene promoter in intron 1 of the ABCA1 gene, which contained putative LXR elements, and to identify a new ABCA1 exon (Exon 1A) located downstream of Exon 1.

Singaraja et al.33 like Cavelier et al.,32 also created ABCA1 transgenic mice using a BAC clone that lacked Exon 1 and its promoter and began 13 kb upstream of Exon 2, but in a mixed genetic background (C57BL/6 × CBAJ). In the transgenic mice created by Singaraja et al.,33 three transcription initiation start sites were found in intron 1, leading to three alternatively spliced transcripts beginning at −2566 (Exon 1d), −2210 (Exon 1c, which corresponded to the Cavelier intron 1 start site), and −1746 (Exon 1b) upstream of Exon 2. These transcription initiation sites were identified in normal human and mouse liver as well as in the liver of transgenic mice. The authors demonstrated that this promoter, which contained several LXR elements, was regulated by sterols, because cholesterol feeding enhanced ABCA1 gene expression. Transgenic mice were shown to have increased expression (>3-fold) of both ABCA1 mRNA and protein in the liver, macrophage, lung, small intestine, stomach, testis, and brain.

To additionally evaluate the effects of ABCA1 overexpression on atherosclerosis, ABCA1-Tg mice have now been crossed with apoE-KO42–43 or LDLr-KO mice.44 The ABCA1-B Tg mouse created by Vaisman et al.,31 already in the atherosusceptible strain C57Bl/6, was crossed with an apoE-KO or LDLr-KO mice to create an ABCA1 × apoE-KO strain or an ABCA1 × LDLr-KO strain in a pure C57Bl/6 background. Singaraja et al.33 crossed their ABCA1 BAC-Tg mice, which began in a C57Bl/6 × CBAJ background, with apoE-KO mice, generating apoE-KO × ABCA1-Tg mice backcrossed to the N6 generation (98.4%) to the C57Bl/6 background.

The Table outlines the various lines of ABCA1-Tg mice and summarizes the effects of ABCA1 overexpression on plasma cholesterol and HDL as well as atherosclerosis in the different lines. The following sections discuss and interpret these results in detail.

Effects of ABCA1 Overexpression on HDL Metabolism

As anticipated from studies in human patients and animal models with mutations in the ABCA1 gene,9,14,18–20,29,30 analysis of ABCA1-Tg mice generated by two different laboratories has demonstrated that ABCA1 overexpression in transgenic mice significantly contributes to the plasma concentrations of HDL-C.31,33

In the ABCA1-Tg mouse models generated by Vaisman et al.,31 overexpression of human ABCA1 in liver (4- and 9-fold) and macrophages (3- and 6-fold) of C57Bl/6 mice raised the plasma HDL-C and apoA-I levels on both chow (1.8- and 1.3-fold) and proatherogenic (2.8- and 2-fold) diets.31,42 The moderate increase in plasma apoA-I levels relative to HDL-C levels in ABCA1-Tg mice suggested the formation of a
Hepatic ABCA1: A Major Source of HDL Cholesterol in Plasma

An interesting possibility that arose during the analysis of plasma lipoproteins in ABCA1-overexpressing mice was that liver may be a major source of HDL-C. In the ABCA1-Tg model generated by Vaisman et al., overexpression of human ABCA1 in only the liver and macrophages significantly raised plasma HDL-C levels. Together with previous reports that demonstrate that the liver is a major site of ABCA1 gene expression and that macrophage ABCA1 does not significantly contribute to plasma HDL levels in mice, these data implicate hepatic ABCA1 as an important source of HDL-C in plasma.

Additional studies support this concept. ABCA1 has recently been localized to the basolateral surface of the polarized WI-B liver cells, indicating that ABCA1 facilitates transport into the plasma rather than the bile. In addition, increased hepatocellular cholesterol has been observed in a patient with Tangier disease and in the liver of the WHAM chicken, consistent with the role of the ABCA1 transporter in modulating intrahepatic cholesterol levels. The contribution of hepatic ABCA1 to plasma HDL-C has been most recently evaluated by systemic infusion of recombinant adenovirus vectors, which target expression of human ABCA1 to the liver of injected mice. Preliminary findings reported by Basso et al. have demonstrated that increased hepatic expression of ABCA1 significantly raises plasma levels of mature, lipidated HDL, providing direct in vivo evidence that ABCA1-mediated cholesterol efflux from the liver is a major source of cholesterol for plasma HDL.

These data indicate that in addition to its known role in the synthesis of apoA-I and formation of nascent HDL, the liver also contributes to the generation of mature, lipidated HDL via ABCA1-mediated hepatic cholesterol efflux. These findings alter the concept of reverse cholesterol transport as a simple one-way movement of cholesterol from peripheral cells to the liver. The physiological implications of the ABCA1-mediated efflux of hepatic cholesterol to plasma HDL are not completely understood. The transport of hepatic cholesterol to plasma HDL by ABCA1 may emerge as an important secretory pathway, in addition to VLDL, for modulating intrahepatic cholesterol concentrations. Liver-derived HDL-C may also serve as a source of cholesterol for steroidogenic tissues and contribute to the nascent HDL pool after remodeling by CETP, LCAT, and lipases. Additional studies will be required to more fully understand the role of ABCA1 in regulating hepatic cholesterol as well as plasma HDL levels.

Effects of ABCA1 Overexpression on the Metabolism of ApoB-Containing Lipoproteins

Studies involving patients with Tangier disease and ABCA1-deficient animal models indicate that in addition to modulating plasma HDL levels, changes in ABCA1 gene expression also alter the plasma concentrations of the apoB-containing lipoproteins. Thus, VLDL-C and LDL-C levels are often but not always decreased in ABCA1 deficiency. Lowering of apoB-containing lipoproteins was even more evident in ABCA1-KO mice crossed into either
apoE-KO or LDLr-KO backgrounds. Kinetic analysis in patients with Tangier disease demonstrated that reduced LDL was attributable to hypercatabolism of abnormal, dense LDL, which the authors attributed to the low levels of HDL and the inability of CETP to appropriately exchange lipids between HDL and the apoB-containing lipoproteins. In addition to LDL hypercatabolism, other processes may also contribute to the lowering of the apoB-containing lipoproteins as a consequence of reduced ABCA1 expression. These combined data indicate that either directly or indirectly, ABCA1 expression alters the metabolism of the apoB-containing lipoproteins.

Analysis of the plasma lipid profile in ABCA1-Tg mice supports a role for ABCA1 expression in modulating apoB-containing lipidoprotein metabolism. Joyce et al reported that the plasma cholesterol concentrations in ABCA1-Tg mice fed a proatherogenic diet for 14 weeks were markedly reduced (−63%), primarily because of lowering of the plasma apoB-containing lipoproteins (−53%) and apoB (−36%). Kinetic studies demonstrated that the reduction of the apoB-containing lipoproteins in these ABCA1-Tg mice generated by Vaismann et al was attributable to enhanced catabolism of LDL, implicating the LDL receptor in facilitating the ABCA1-mediated reduction in these proatherogenic lipoproteins. Consistently, subsequent studies of LDLr-KO mice on a regular chow diet overexpressing ABCA1 in liver and macrophages found proatherogenic increases in the plasma lipid profile, including significantly higher plasma cholesterol and non–HDL-C, with marked accumulation (2- to 3-fold) of the cholesterol in the apoB-containing lipoproteins, as well as increased levels of apoB and apoE. It should be noted that in the ABCA1-Tg mice generated by Singaraja et al, cholesterol feeding increased the plasma total cholesterol levels (15%), mostly attributable to increased cholesterol in the apoB-containing lipoproteins (15%). Although these changes are less pronounced than those observed by Joyce et al, these BAC ABCA1-Tg mice had been fed a proatherogenic diet for only 7 days.

Taken together with the Tangier disease metabolic studies, these findings suggest that changes in ABCA1-mediated cholesterol efflux can alter the lipid content and composition not only of HDL but also of the apoB-containing lipoproteins, which in turn changes the catabolism of these particles. However, the mechanism by which ABCA1 alters the plasma concentrations of the apoB-containing lipoproteins is likely to be more complex than ABCA1-induced changes in particle catabolism. Alterations in cholesterol transport resulting in increased ABCA1 gene expression may also result in compensatory changes in enzymes and receptors that modulate apoB synthesis and catabolism.

**Effect of ABCA1 Overexpression on Atherosclerosis**

Joyce et al provided the first in vivo evidence to support an antiatherogenic role for ABCA1 overexpression in transgenic mice. Overexpression of human ABCA1 in the liver and macrophages of C57Bl/6 mice on a proatherogenic diet markedly reduced aortic atherosclerosis by 65%. In these mice, in addition to facilitating the cellular efflux of cholesterol and thus preventing cholesterol accumulation and foam cell formation in macrophages, increased ABCA1 raised the plasma HDL-C levels and lowered the plasma concentrations of the proatherogenic, apoB-containing plasma lipoproteins. These combined effects explain the marked reduction in aortic atherosclerosis of ABCA1-Tg mice.

However, ABCA1 overexpression in an apoE-KO background has resulted in conflicting data. Joyce et al reported a significant increase (2-fold) in atherosclerosis in ABCA1-Tg×apoE-KO mice. In these animals, enhanced expression of the human ABCA1 gene in liver (9-fold) and macrophages (6-fold) was still evident, but ABCA1 overexpression did not raise plasma HDL-C concentrations. Although a trend toward increased plasma total cholesterol and non–HDL-C levels was noted, Joyce et al reported no significant changes in the plasma lipid profile in ABCA1-Tg×apoE-KO mice. These findings indicate that, by mechanisms that are not presently understood, apoE may enhance ABCA1-mediated cholesterol efflux from cells.

Singaraja et al reported that their ABCA1 BAC-Tg×apoE-KO mice showed a very modest significant increase in plasma levels of cholesterol (1.15-fold), attributable to increased non–HDL-C (1.15-fold) and HDL-C (1.2-fold), thus reinforcing the trend toward increased plasma total cholesterol and non–HDL-C observed by Joyce et al. In addition, plasma apoB but not apoA-I levels were increased by 1.18-fold. Macrophages from these animals had increased (+38%) cellular cholesterol efflux compared with apoE-KO mice. However, despite the proatherogenic changes in the lipid profile of their BAC ABCA1-Tg mice, Singaraja et al reported a 2.6-fold reduction in aortic atherosclerosis. Lesions of the BAC ABCA1-Tg×apoE-KO mice lacked a fibrous cap and were much less developed than lesions of the apoE-KO controls.

The reason for the difference in atherosclerosis between the ABCA1-Tg×apoE-KO mouse lines by Singaraja et al and Joyce et al is not presently understood. However, the two mouse lines differ in the constructs, tissue expression sites, and levels of ABCA1 gene expression. Joyce et al overexpressed ABCA1 in liver and macrophages by placing ABCA1 cDNA under control of the apoE promoter, which confers sterol regulation as well as liver and macrophage overexpression. In these mice, ABCA1 was not overexpressed in any other tissue. In contrast, Singaraja et al used a BAC construct in which the ABCA1 gene was under control of the ABCA1 intron 1 promoter but was missing naturally occurring up-stream sequences that included the Exon 1 promoter as well as Exon 1. In these BAC-ABCA1-Tg×apoE-KO mice, ABCA1 was overexpressed not just in liver and macrophages but also in kidney, brain, aorta, gonads, and large intestine; higher levels of ABCA1 expression were present in the gonads and kidneys than in liver and macrophages. Interestingly, this pattern of gene expression differs from that reported by Cavelier et al, who used a construct (BAC1) that contained both Exon 1 and intron 1 promoters as well as naturally occurring up-stream sequences. The Cavelier BAC1 ABCA1-Tg mice, with ABCA1 expression controlled by a more complete and more physiological ABCA1 promoter than the Singaraja ABCA1 BAC-Tg×apoE-KO mice, had higher expression levels of ABCA1 primarily in liver and

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macrophages. Other differences between the two studies include the genetic background of the mice and the number and sex of the mice used for the analysis of aortic atherosclerosis. Joyce et al compared the aortic atherosclerosis of a large number of male and female ABCA1 knockout mice generated in a pure C57Bl/6 background with apoE-KO mice of the identical background strain. In contrast, Singaraja et al compared atherosclerosis of BAC-ABCA1-Tg × apoE-KO mice (n = 12) to control apoE-KO littermates (n = 7). These mice, whose sex was not reported, originated from a mixed C57Bl/6 × CBAJ background and were backcrossed to the N6 generation (98.4%) to the C57Bl/6f background. Additional studies will be required to fully understand the reasons for the different atherogenic outcomes in the two ABCA1-Tg × apoE-KO mouse models.

Analysis of aortic atherosclerosis in ABCA1-KO mice, similar to studies in ABCA1-Tg mice, has also provided unexpected findings. Despite marked changes in the plasma lipoprotein profile and macrophage cholesterol efflux, the anticipated increase in atherosclerosis in ABCA1-KO mice was not observed. However, selective inactivation of ABCA1 in macrophages markedly enhanced aortic atherosclerosis in apoE-KO and LDLr-KO mice. These findings indicate that although ABCA1-mediated cholesterol efflux in macrophages reduces atherosclerosis, this beneficial, antiatherogenic function of ABCA1 may be overwhelmed by ABCA1-mediated proatherogenic changes in the plasma lipoproteins and perhaps by other unrecognized ABCA1 functions. Hence, ABCA1-induced changes in macrophage cholesterol efflux do not always predict atherogenic outcome. The combined results of studies in ABCA1-KO and ABCA1-Tg mice indicate that the effects of ABCA1 on atherosclerosis are complex and highly variable. Additional characterization of the different transgenic mice and knockout animal models may help to additionally clarify the important role of ABCA1 in atherosclerosis.

Summary

The discovery of ABCA1 as a key transporter that facilitates cellular cholesterol efflux has generated considerable interest in evaluating its potential role as an antiatherogenic agent. Analysis of transgenic mice that overexpress ABCA1 has shown that increased ABCA1 raises plasma HDL levels, increases cholesterol efflux from macrophages, and reduces diet-induced atherogenic changes in different mouse models. In addition, analysis of ABCA1-Tg mice has provided additional evidence that ABCA1 alters the plasma levels of the proatherogenic apoB-containing lipoproteins, indicating an integrated compensatory response in the plasma lipoproteins in response to changes in ABCA1 expression. Analysis of mice with enhanced hepatic expression of ABCA1 has substantiated the role of the liver as an important source of plasma HDL-C and has identified a potential new secretory pathway for modulating intracellular cholesterol concentrations. The discordant results obtained by different laboratories on the development of atherosclerosis in the ABCA1-Tg × apoE-KO mice illustrate the complexity of the cellular pathways of cholesterol metabolism and the factors modulating the development of atherosclerosis. Further studies in additional animal models will be necessary to fully understand the role of ABCA1 in atherosclerosis within the framework of other genetic defects in lipoprotein metabolism. The combined results of in vitro and in vivo studies seem to validate the selection of ABCA1 as a potential therapeutic target for the development of pharmacological agents for treatment of low HDL syndromes and cardiovascular disease.

References


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