Targeted Disruption of the PDZK1 Gene in Mice Causes Tissue-specific Depletion of the High Density Lipoprotein Receptor Scavenger Receptor Class B Type I and Altered Lipoprotein Metabolism*

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PDZK1, a multi-PDZ domain containing adaptor protein, interacts with various membrane proteins, including the high density lipoprotein (HDL) receptor scavenger receptor class B type I (SR-BI). Here we show that PDZK1 controls in a tissue-specific and post-transcriptional fashion the expression of SR-BI in vivo. SR-BI protein expression in PDZK1 knock-out (KO) mice was reduced by 95% in the liver, 50% in the proximal intestine, and not affected in steroidogenic organs (adrenal, ovary, and testis). Thus, PDZK1 joins a growing list of adaptors that control tissue-specific activity of cellular surface receptors. Hepatic expression of SR-BI, a minor splice variant with an alternative C-terminal cytoplasmic domain, was not affected in PDZK1 KO mice, suggesting that binding of PDZK1 to SR-BI is required for controlling hepatic SR-BI expression. The loss of hepatic SR-BI was the likely cause of the elevation in plasma total and HDL cholesterol and the increase in HDL particle size in PDZK1 KO mice, phenotypes similar to those observed in SR-BI KO mice. PDZK1 KO mice differed from SR-BI KO mice in that the ratio of unesterified to total plasma cholesterol was normal, females were fertile, and cholesteryl ester stores in steroidogenic organs were essentially unaffected. These differences may be due to nearly normal extrahepatic expression of SR-BI in PDZK1 KO mice. The PDZK1-dependent regulation of hepatic SR-BI and, thus, lipoprotein metabolism supports the proposal that this adaptor may represent a new target for therapeutic intervention in cardiovascular disease.

Cytoplasmic adaptor proteins that bind directly to cell surface receptors or to receptor-associated proteins play crucial roles in regulating various biological processes including signal transduction, adhesion, membrane trafficking, and cellular transport (1). They usually consist of evolutionarily shuffled combinations of modular protein interaction domains such as src-homology (SH2, SH3), phosphotyrosine binding, and PDZ domains that recognize short peptide or phosphopeptide epitopes (e.g. PDZ domains usually bind to the C-terminal 3–4 residues of interacting proteins) (2). These adaptors help link one or more integral membranes and otherwise non-membraneous proteins into functional complexes (1). For example, PDZK1, a four PDZ domain-containing protein whose expression is increased in a significant number of human kidney, colon, lung, and breast carcinomas, interacts with a number of membrane-associated transporter proteins, including cMOAT/ MRP2 (3), the multidrug resistance-associated protein, the type IIa Na+/P+ cotransporter (4), the chloride channel CIC-3B (5), the cystic fibrosis transmembrane conductance regulator (5, 6), and the high density lipoprotein (HDL) receptor called scavenger receptor class B type II (SR-BII) (7, 8). The multiple PDZ domains in PDZK1 may mediate its simultaneous interaction with several target proteins and, thus, permit it to orchestrate complex biological functions by acting as a scaffolding protein (9).

Recently the phosphotyrosine binding domain-containing adaptor ARH (product of the defective gene in autosomal recessive hypercholesterolemia (10)) was shown to bind to the LDL receptor and components of the clathrin endocytic machinery and to control LDL receptor endocytic activity in selective cell types (hepatocytes and lymphocytes but not fibroblasts) (11, 12). In mice and humans, ARH deficiency causes loss of hepatic LDL receptor endocytic activity and, consequently, hypercholesterolemia (13). The cell type-specific requirement for ARH suggests that the activities of other adaptors can substitute for that of ARH in some cells (e.g. fibroblasts), that these alternative adaptors are not expressed in or do not function effectively in ARH-sensitive cells (e.g. hepatocytes and/or lymphocytes), or that ARH plays some as yet undefined critical and dominant roles in controlling the endocytosis of LDL receptor in ARH-sensitive but not ARH-insensitive cells. These observations also raise the possibility that other adaptor proteins might control the function of other

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† The abbreviations used are: HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; SR-BI and SR-BII, scavenger receptor class B types I and II, respectively; ARH, autosomal recessive hypercholesterolemia; KO, knock-out.
cell surface receptors in a similarly tissue-specific fashion. Manipulation of the activities of such adaptors might provide new insights into receptor physiology (e.g. new approach for tissue-specific knock-out/knockdown of receptor activity) and possibly attractive targets for pharmaceutical intervention in related disease processes (e.g. atherosclerosis) (12).

Here we have used recently described homoygous null PDZK1 knock-out (KO) mice (14) to show that PDZK1 controls, in a tissue-specific, post-transcriptional fashion, the expression of the HDL receptor SR-BI and HDL metabolism. In PDZK1 KO mice SR-BI expression was dramatically reduced in the liver, mildly decreased in the proximal intestine, and not affected in the steroidogenic organs (adrenal, ovary, and testis). The loss of hepatic SR-BI expression in these mice is the likely cause of the elevation in total plasma cholesterol levels (14) and the presence of abnormally large HDL particles, as in the case of the HDL receptor SR-BI and HDL metabolism. In PDZK1 KO mice SR-BI expression was dramatically suppressed by transgenic hepatic overexpression of the small PDZK1-interacting protein MAP17 (3, 9, 17, 18). However, PDZK1 KO mice differ from SR-BI KO mice in a number of ways. First, in contrast to the presence of excess unesterified cholesterol in the plasma of SR-BI KO mice (42), the unesterified-to-total cholesterol ratio was normal in PDZK1 KO mice. Second, the steroidogenic tissues of PDZK1 KO mice appeared to contain normal cholesteryl ester stores, presumably because these tissues expressed normal amounts of SR-BI protein. Finally, female PDZK1 KO mice, but not SR-BI KO females, are fertile (14, 15). Thus, PDZK1 joins ARH in which it is likely to be a growing family of adaptors that control the tissue-specific expression and activity of cell surface receptors. Our findings also indicate that PDZK1 plays a key role in the control of HDL metabolism through its interaction with SR-BI in the liver and suggest that PDZK1 KO mice might be useful surrogates for mice with liver-specific (e.g. cre/lox) ablation of SR-BI expression for the analysis of the role of hepatic SR-BI expression in lipoprotein metabolism.

EXPERIMENTAL PROCEDURES

Animals—PDZK1 knock-out and wild-type mice (129SvEv background) were maintained on a normal chow diet (14) and were 6–12 weeks old at the time of the experiments. All procedures were performed in accordance with the Beth Israel Deaconess Medical Center guidelines.

Immunoblot Analysis, Immunoperoxidase, and Oil Red O Studies—Antibodies against SR-BI, SR-BII, and actin were purchased from Biodesign (Saco, ME) and antiserum against apolipoprotein A-I (apoA-I) was purchased from R. L. Raffai and K. H. Weisgraber (University of California San Francisco, CA). Anti-apolipoprotein E (apoE) antiserum was a gift described (19, 20). Anti-apolipoprotein E (apoE) antisera was a gift from R. L. Raffai and K. H. Weisgraber (University of California San Francisco, CA), and antisera against apolipoprotein A-1 (apoA-I) was purchased from Bodesign (Saco, ME), Karen Kozarsky (GlaxoSmithKline) provided the Kk-B-1 antibody that recognizes the extracellular domains of both SR-BI and SR-BII.

For immunoperoxidase studies tissues were fixed for 4 h in 4% paraformaldehyde in phosphate-buffered saline, pH 7.4, at 4 °C and then transferred to 30% sucrose in phosphate-buffered saline, pH 7.4, overnight at 4 °C. Tissues were then frozen in OCT compound (Miles Diagnostics, Elkhart, IN) and stored in liquid nitrogen. Immunoperoxidase studies were performed on 5-μm fixed-frozen tissue sections using primary antibodies against SR-BI. Normal rabbit IgG was used as negative controls. The sections were then incubated with a biotinylated anti-rabbit IgG using a 1/200 dilution (Vector, Burlingame, CA) and subsequently treated with the Vectastain ABC reagents (Vector) and diaminobenzidine (Research Genetics, Inc., Huntsville, AL), according to the manufacturer’s protocol. Oil Red O and hematoxylin stainings were performed on 6-μm unfixed frozen tissue sections as previously described (21).

Reverse Transcription—Total RNA was reverse-transcribed using the ABI Prism 7700 sequence detection system and TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA). Each reaction contained 1X reverse transcription buffer, 5.5 mM MgCl₂, 50 μM each dNTP, 2.5 μM random hexamer, 0.4 μM of RNAse inhibitor, 1.25 units of multiscribe reverse transcriptase, 0.1 μg of total RNA, and RNAse-free distilled H₂O to a total volume of 10 μl per reaction. Reverse transcription reaction cycle parameters were as follows: 10 min at 25 °C, 30 min at 48 °C, and 5 min at 95 °C.

SYBR Green Real-time Quantitative PCR—Oligonucleotide primers were designed using Primer Express software version 1.5 based on gene sequences obtained from the National Center for Biotechnology Information (NCBI) database. All reactions were performed using an ABI Prism 7700 sequence detection system (Applied Biosystems). Reactions were carried out in duplicate in a 50-μl reaction volume containing reverse-transcribed cDNAs, 25 μl of 2X SYBR Green Master Mix, and each forward and reverse primer at a concentration of 50 nm. Conditions for all SYBR Green PCR reactions were 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. Data were gathered and analyzed using the SDS 1.9 software on a Power Macintosh computer (Apple Computer, Cupertino, CA). Results were normalized with respect to glyceraldehyde-3-phosphate dehydrogenase expression. Threshold cycle (Ct) values were exported into a Microsoft Excel worksheet for calculation of gene expression according to the ΔΔCt method (Applied Biosystems). Levels of gene expression in knock-out mice were represented with respect to those of wild-type animals as knock-out/wild-type ratios.

Plasma Cholesterol and Lipoprotein Analysis—Plasma was collected from PDZK1 knock-out and wild-type mice after a 4-h fast. Total cholesterol, unesterified (“free”) cholesterol, triglycerides, and phospholipids were measured using kits from Wako (Richmond, VA). Plasma was fractionated by fast protein liquid chromatography, and the cholesterol and phospholipid composition of the fractions were determined by enzymatic kit and immunoblotting, respectively (15).

Statistical Analysis—Data are shown as the means ± S.E. Statistically significant differences were determined by pairwise comparisons of each value from knock-out mice with wild-type controls by using unpaired Student’s t test. p values < 0.05 were considered to be statistically significant for differences between experimental groups.

RESULTS

We have previously reported that PDZK1 KO mice exhibit an ~1.8-fold elevation in plasma total cholesterol concentration (14), and others have suggested that PDZK1 interactions with SR-BI in the liver may play an important role in controlling SR-BI expression and function and, thus, HDL metabolism (7, 8, 16, 18). Because the loss of SR-BI expression in SR-BI KO mice results in an ~2-fold elevation in plasma cholesterol (15), it seemed possible that the increased plasma cholesterol in PDZK1 KO mice might have been due to reduced hepatic SR-BI protein levels in these mice. We therefore used immunoblot and immunohistochemical analyses to measure SR-BI protein expression in PDZK1 KO mice.

The immunoblot analysis of SR-BI expression (Fig. 1 and data not shown) demonstrates that there was an ~95% reduction in SR-BI protein expression in the livers of PDZK1 KO mice relative to wild-type controls. In contrast, there was only a modest reduction (50%) in the much lower expression levels
of SR-BI protein in the small intestine and no detectable change in the adrenal gland, which exhibits especially high SR-BI levels (19). The results of immunoblotting were supported by immunohistochemical (immunoperoxidase) analysis of SR-BI expression (Fig. 2) in the liver (hepatocytes), small intestine (epithelial cells), adrenal gland (cortex), testis (Leydig cells), and ovary (stroma and corpus luteum) in control wild-type (left) and PDZK1 KO (right) mice. The cell type and intracellular patterns of SR-BI expression in these organs were similar to those reported previously (19, 22, 23). The intensity of SR-BI staining in PDZK1 KO mice relative to wild-type mice was markedly reduced in liver, moderately reduced in small intestinal mucosa, and not changed in adrenal cortical, ovary, or testis. The ~50% reduction in SR-BI protein expression in the small intestine (Fig. 1) was apparently due to a homogeneous decrease in all SR-BI-expressing epithelial cells of the mucosa (Fig. 2) rather than to varying extents of altered SR-BI expression in different subtypes of cells within this organ.

The expression of SR-BI in steroidogenic tissues has been shown to be essential for the normal accumulation of stored cholesteryl esters in cytoplasmic lipid droplets (15, 21). When we visualized the neutral lipid stores in the adrenals by staining tissue sections with the dye Oil Red O we saw essentially no differences in staining between the wild-type and PDZK1 KO samples (Fig. 3). Thus, in steroidogenic tissues, in which PDZK1 expression is low (7, 9) and SR-BI expression is normally high (19), SR-BI protein was expressed in normal amounts and distribution and apparently functioned properly in providing substrate for cholesteryl ester storage even in the absence of a functional PDZK1 gene.

The expression of SR-BI in the liver is generally thought to be responsible for SR-BI marked influence on the structure and abundance of HDL in murine plasma (15). In homozygous null SR-BI KO relative to wild-type mice, HDL cholesterol levels and the sizes of HDL particles are increased based on gel filtration chromatography analysis. The abnormally large HDL particles in SR-BI KO mice also carry increased amounts of apoE (15). Thus, if the loss of hepatic SR-BI protein expression in PDZK1 KO mice is responsible for their hypercholesterolemia, we would expect that the plasma lipoprotein profiles in these mutant mice might in some ways resemble the abnormal lipoproteins found in SR-BI KO mice.

Table I shows the lipid composition of plasma from wild-type and PDZK1 KO male and female mice. The effects of the mutation on the plasma lipids of males and females were generally similar, and the pooled results will be considered below. As previously reported (14), the PDZK1 KO mice were hypercholesterolemic. The total plasma cholesterol was ~1.7-fold higher in the PDZK1 KO mice; the fold increases in unesterified and esterified cholesterol were 1.9 and 1.6, respectively. The in increases in plasma cholesterol were accompanied by increases in phospholipids and triglycerides. To further assess the structures of the lipoproteins, plasma samples from individual mice were size-fractionated by Sepharose 4B fast protein liquid chromatography size exclusion chromatography. The lipoprotein total cholesterol profiles of representative animals are shown in Fig. 4A.

As previously reported, most of the plasma cholesterol in wild-type mice was in HDL-size particles centered at fraction...
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Effects of disruption of the PDZK1 gene on plasma lipid concentrations

All animals were 8 weeks old and were fasted for 4 h before analyses of plasma lipids. Values are expressed as the mean ± S.E. TC, total cholesterol; UC, unesterified cholesterol; TG, triglycerides; PL, phospholipids; WT, wild type; M, males; F, females.

| Gender | Genotype | TCa | UCa | UC/TC | TGb | PLc | Sample size |
|--------|----------|-----|------|--------|------|------|-------------|
| Males  | WT       | 91 ± 7 | 22 ± 1 | 0.42 ± 0.01 | 32 ± 7 | 164 ± 9 | n = 7 |
|        | PDZK1 KO | 144 ± 8 | 38 ± 2 | 0.27 ± 0.01 | 36 ± 5 | 212 ± 12 | n = 6 |
| Females | WT       | 78 ± 4 | 18 ± 1 | 0.23 ± 0.01 | 16 ± 1 | 131 ± 6 | n = 8 |
|        | PDZK1 KO | 139 ± 6 | 34 ± 1 | 0.24 ± 0.004 | 25 ± 3 | 188 ± 5 | n = 8 |
| M + F  | WT       | 84 ± 4 | 19 ± 1 | 0.23 ± 0.01 | 24 ± 4 | 146 ± 7 | n = 15 |
|        | PDZK1 KO | 141 ± 5 | 36 ± 1 | 0.25 ± 0.01 | 30 ± 3 | 198 ± 6 | n = 14 |

a p < 0.001 vs. the corresponding wild-type group.
b p < 0.001 vs. the corresponding wild-type group.
c p < 0.01 vs. the corresponding wild-type group.
d p < 0.01 vs. the corresponding wild-type group.

Fig. 4. Fast protein liquid chromatography profiles of plasma lipoprotein cholesterol (A) and apolipoproteins (B) for wild-type and PDZK1 KO mice. A, the chromatograms represent analyses of the total cholesterol content of fractions from individual 4-h fasted wild-type (filled circles) and PDZK1 KO (open circles) mice and are representative of multiple, independent determinations. Approximate positions of VLDL, IDL/LDL, and HDL elution are indicated by brackets and were determined as previously described (15). B, combined immunoblot analyses of fractions 15–40 from the chromatograms shown in A were performed with polyclonal antibodies to apoE, and apoA-I (top panels, wild-type mouse; bottom panels, PDZK1 KO mouse). Separate gels and membranes were used for fractions 15–28 (left) and fractions 28–41 (right). Fraction 28 of each plasma sample was loaded on both gels to allow comparison between blots.

SR-BI protein expression is the most likely cause of the hypercholesterolemia in PDZK1 KO mice.

We observed two striking unanticipated differences between PDZK1 KO and SR-BI KO mice. In wild-type mice on a mixed C57/Bl6:129 genetic background, the unesterified to total cholesterol ratio is about 0.26–0.31, whereas that for SR-BI KO mice is about 0.5 (24, 25). There was, however, no statistically significant difference in this ratio between wild-type and PDZK1 KO mice (Table I). Another difference between SR-BI KO and PDZK1 KO mice was that female SR-BI KO mice are sterile (15), whereas PDZK1 KO mice were fertile, producing an average of 7 pups/month/mother.

To explore the mechanism by which PDZK1 influences SR-BI protein expression in a tissue-specific manner, we used reverse transcription-PCR to measure the relative levels of SR-BI mRNA in several tissues in PDZK1 KO and wild-type control mice. Table II shows that there were no significant differences in the SR-BI mRNA levels in the livers, small intestines, or steroidogenic tissues (adrenal, testis, and ovary) of these mice. Thus, PDZK1-dependent regulation of hepatic and intestinal SR-BI protein expression is due to a post-transcriptional mechanism.

It seemed likely that the binding of PDZK1 to the C terminus of SR-BI (7, 8) is directly responsible for maintaining normal levels of hepatic and intestinal SR-BI protein levels in wild-type mice. To begin to examine the role of the C terminus of SR-BI in determining its hepatic PDZK1 sensitivity, we evaluated the hepatic levels of SR-BI in these animals. SR-BI is a normally minor protein product of the SR-BI gene that arises from alternative RNA splicing (26, 27). It has been estimated that SR-BII composes about 12% of the total SR-BI plus SR-BII protein in murine liver and ~5% in the testes and adrenal glands (26). SR-BII differs from SR-BI only in that the 42 most C-terminal residues in the 47-residue C-terminal cytoplasmic domain of SR-BI are replaced in SR-BII by 39 residues encoded by an alternatively spliced exon. The altered C terminus in SR-BII renders this protein less efficient at mediating selective cholesteryl ester uptake from HDL (26). Unlike the four C-terminal residues in murine SR-BI (EAKL), those in SR-BII (SAMA) are not expected to bind to PDZ domains (2). Indeed, Fig. 5 shows that when we used SR-BI (left)- and SR-BII (middle)-specific antibodies we observed no difference in the immunodetectable levels of SR-BII in the livers of PDZK1 KO and wild-type mice despite the marked difference for SR-BI. As expected from the normally low abundance of SR-BII in the liver, when we used an antibody that recognizes the extracellular domains of both SR-BI and SR-BII (Fig. 5, right), the immunoblotting result was similar to that with the SR-BI-specific antibody. These results strongly suggest that the PDZK1 sensitivity of hepatic SR-BI is due at least in part to its
mice bear some resemblance to those seen in SR-BI KO mice like particles. The large HDL-like particles in the PDZK1 KO accompanied by the presence of abnormally large plasma HDL-hepatic SR-BI protein expression in PDZK1 KO mice that was expression. It seemed possible that the hypercholesterolemia in PDZK1 KO mice that exhibit an elevation in plasma total cholesterol concentration (14), it is the case of hypercholesterolemia. This seems likely that PDZK1 normally stabilizes, by as yet unknown mechanisms, SR-BI by binding directly to the C terminus.

PDZK1 has been virtually eliminated in the livers of mice using three distinct approaches, 1) disruption of the PDZK1 gene (Ref. 14 and this study), 2) treatment with ciprofibrate (16), and 3) hepatic overexpression of the PDZK1 binding Protein MAP17 (17, 18, 36). In all three systems the loss of hepatic PDZK1 was accompanied by a dramatic reduction of hepatic SR-BI protein expression and the appearance of large HDL-like particles, which in some cases were shown to be enriched in apoE. Some of the effects of hepatic PDZK1 ablation on plasma lipoproteins resemble those in SR-BI KO mice. Thus, hepatic PDZK1 acting through SR-BI helps control murine lipoprotein metabolism. The roles of reduced PDZK1 expression and consequent alterations in SR-BI protein expression and lipoprotein metabolism in other experimental systems (37–40) remains to be explored.

There are two features of lipoprotein metabolism that differ between SR-BI KO mice and those with hepatic PDZK1 ablation. First, the leftward shift in the HDL peak in the lipoprotein profiles (larger HDL-like particles) is not as dramatic in the hepatic PDZK1 ablation models. Second, the high ratio of plasma unesterified to total cholesterol (0.5 versus the normal values of 0.2–0.3) found in the SR-BI KO mice (24, 25) has not been observed in the PDZK1 KO or ciprofibrate-treated animals. Detailed compositional data for plasma lipoproteins has not yet been reported for the MAP17 transgenic mice (18). There are several potential explanations for the differences between SR-BI KO mice and the murine models of decreased hepatic PDZK1 expression. First, they may be due to residual expression of hepatic SR-BI in the PDZK1 ablation models (for example, 5% PDZK1), although SR-BI could not be detected in the livers of ciprofibrate-treated mice (16), suggesting that residual receptor levels were extremely low. Second, the SR-BI splice variant is expressed at normal levels in PDZK1 KO mice. SR-BI would not be expressed in SR-BI KO mice due to the nature of the targeted mutation in those animals (15). The low level expression (Refs. 26 and 27 and this study) of hepatic SR-BI and its relatively low selective uptake efficiency compared with SR-BI (4-fold lower (26)) are not expected to fully substitute for SR-BI function, but may mitigate the loss of hepatic SR-BI on lipoprotein metabolism. However, hepatic SR-BI protein expression levels are significantly lower in ciprofibrate-treated mice than in untreated controls. Thus, differences in hepatic SR-BI expression may not necessarily account for the differences in lipoprotein metabolism observed between PDZK1 and SR-BI knocked-out mice. A third explanation is that the normal or near normal expression of SR-BI in

| Tissue | Fold change (PDZK1 KO/wild-type) |
|--------|---------------------------------|
| Liver  | 1.02 ± 0.27                    |
| Adrenal| 1.04 ± 0.14                    |
| Testes | 0.98 ± 0.11                    |
| Ovary  | 0.84 ± 0.29                    |
| Small intestine | 0.98 ± 0.10   |

Fig. 5. Immunoblot analysis of SR-BI and SR-BII expression in livers from wild-type and PDZK1 knock-out mice. Liver lysates from wild-type (WT) and PDZK1 KO mice were subjected to immuno- blotting analysis using either anti-SR-BI-specific (left) or anti-SR-BII-specific (center) antibodies or an antibody that can recognize both forms of the receptor (right).

C-terminal cytoplasmic domain and that direct interactions of SR-BI and PDZK1 are most likely responsible for SR-BI sensitivity to PDZK1 deficiency in the liver. These results and conclusions are consistent with the previous findings that deletion of the C terminus of SR-BI prevents its association with PDZK1 in cultured cells (7, 8) and prevents its expression on the surface of murine hepatocytes in vivo (8).

DISCUSSION

The HDL receptor SR-BI controls the structure and metabolism of HDL and protects against atherosclerosis (24). Its athero-protective effects may be due to its participation in “reverse cholesterol transport.” Reverse cholesterol transport is the HDL-mediated transport of cholesterol from peripheral tissues (including atherosclerotic plaques) to the liver and then to the bile for excretion (28–30). SR-BI delivers cholesterol esters and other lipids from HDL to cells via selective lipid uptake (19, 30–33), a process distinct from classic receptor-mediated endocytosis (34). During selective uptake, HDL binds to SR-BI, transfers its cholesteryl esters to the cell, and then the lipid-depleted HDL dissociates from the cells. Homozygous null SR-BI KO mice are hypercholesterolemic (2-fold elevation), and their HDL particles are abnormally large (15) due to the loss of SR-BI-mediated selective uptake. SR-BI can also mediate unesterified cholesterol efflux from cells to HDL (35), although the physiologic significance of this activity is not clear.

Ikemoto et al. (7) show that the PDZK1 adaptor protein can bind via its N-terminal PDZ domain to the C terminus of SR-BI in vitro and in vivo and speculated that PDZK1 contributes to the proper sorting and delivery of SR-BI to the plasma membranes in hepatocytes as well as to its stability and function in the liver. Mardones et al. (16) and Silver et al. (8, 18) provide indirect support for the suggestion that interactions of SR-BI with PDZK1 might be required for stable hepatic expression of the SR-BI protein. Because PDZK1 KO mice exhibit an ~1.7-fold elevation in plasma total cholesterol concentration (14), it seemed possible that the hypercholesterolemia in PDZK1 KO mice might have been due to a loss in hepatic SR-BI protein expression.

Here we have shown that there was a profound reduction in hepatic SR-BI protein expression in PDZK1 KO mice that was accompanied by the presence of abnormally large plasma HDL-like particles. The large HDL-like particles in the PDZK1 KO mice bear some resemblance to those seen in SR-BI KO mice and other mice in which hepatic PDZK1 expression has been suppressed (Refs. 16 and 18 and see below). Although the levels of hepatic SR-BI protein were very low, the levels of SR-BI mRNA in the livers of PDZK1 KO mice were normal, suggesting a post-transcriptional dependence of SR-BI expression on PDZK1. Unlike the case with SR-BI, there was no loss of SR-BII in the livers of PDZK1 KO mice. SR-BII differs from SR-BI only in its C-terminal cytoplasmic domain (product of alternative splicing from the same gene) (26, 27). The four C-terminal residues in murine SR-BII (SAMA) differ from those in SR-BI (EAKL) and, thus, may not bind to PDZ domains (2). We conclude that PDZK1 is necessary for the normal expression of SR-BI protein in murine liver and that the loss of hepatic SR-BI expression in PDZK1 KO mice is the likely cause of their hypercholesterolemia. It seems likely that PDZK1 normally stabilizes, by as yet unknown mechanisms, SR-BI by binding directly to its C terminus.

There are two features of lipoprotein metabolism that differ between SR-BI KO mice and those with hepatic PDZK1 ablation. First, the leftward shift in the HDL peak in the lipoprotein profiles (larger HDL-like particles) is not as dramatic in the hepatic PDZK1 ablation models. Second, the high ratio of plasma unesterified to total cholesterol (0.5 versus the normal values of 0.2–0.3) found in the SR-BI KO mice (24, 25) has not been observed in the PDZK1 KO or ciprofibrate-treated animals. Detailed compositional data for plasma lipoproteins has not yet been reported for the MAP17 transgenic mice (18). There are several potential explanations for the differences between SR-BI KO mice and the murine models of decreased hepatic PDZK1 expression. First, they may be due to residual expression of hepatic SR-BI in the PDZK1 ablation models (e.g., 5% in PDZK1 KO) although SR-BI could not be detected in the livers of ciprofibrate-treated mice (16), suggesting that residual receptor levels were extremely low. Second, the SR-BI splice variant is expressed at normal levels in PDZK1 KO mice. SR-BII would not be expressed in SR-BI KO mice due to the nature of the targeted mutation in those animals (15). The low level expression (Refs. 26 and 27 and this study) of hepatic SR-BII and its relatively low selective uptake efficiency compared with SR-BI (4-fold lower (26)) are not expected to fully substitute for SR-BI function, but may mitigate the loss of hepatic SR-BI on lipoprotein metabolism. However, hepatic SR-BII protein expression levels are significantly lower in ciprofibrate-treated mice than in untreated controls. Thus, differences in hepatic SR-BII expression may not necessarily account for the differences in lipoprotein metabolism observed between PDZK1 and SR-BI knock-out mice. A third explanation is that the normal or near normal expression of SR-BI in

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