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Serine palmitoyl-CoA transferase (SPT) deficiency and sphingolipid levels in mice

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Abstract

Sphingolipids play a very important role in cell membrane formation, signal transduction, and plasma lipoprotein metabolism, and all these functions may have an impact on atherosclerotic development. Serine palmitoyl-CoA transferase (SPT) is the key enzyme in sphingolipid biosynthesis. To evaluate in vivo SPT activity and its role in sphingolipid metabolism, we applied homologous recombination to embryonic stem cells, producing mice with long chain base 1 (Sptlc1) and long chain base 2 (Sptlc2), two subunits of SPT, gene deficiency. Homozygous Sptlc11 and Sptlc2 mice are embryonic lethal, whereas heterozygous versions of both animals ($Sptlc1^{+/-}$, $Sptlc2^{+/-}$) are healthy. Analysis showed that, compared with WT mice, $Sptlc1^{+/-}$ and $Sptlc2^{+/-}$ mice had: (1) decreased liver Sptlc1 and Sptlc2 mRNA by 44% and 57% ($P<0.01$ and $P<0.0001$, respectively); (2) decreased liver Sptlc1 mass by 50% and Sptlc2 mass by 70% ($P<0.01$ and $P<0.01$, respectively), moreover, Sptlc1 mass decreased by 70% in $Sptlc2^{+/-}$ mouse liver, while Sptlc2 mass decreased by 53% in $Sptlc1^{+/-}$ mouse liver ($P<0.001$ and $P<0.01$, respectively); (3) decreased liver SPT activity by 45% and 60% ($P<0.01$, respectively); (4) decreased liver ceramide (22% and 39%, $P<0.05$ and $P<0.01$, respectively) and sphingosine levels (22% and 31%, $P<0.05$ and $P<0.01$, respectively); (5) decreased plasma ceramide (45% and 39%, $P<0.01$, respectively), sphingosine-1-phosphate (31% and 32%, $P<0.01$, respectively) and sphingosine levels (22.5% and 25%, $P<0.01$, respectively); (6) dramatically decreased plasma lysosphingomyelin (17-fold and 16-fold, $P<0.0001$, respectively); and (7) no change of plasma sphingomyelin, triglyceride, total cholesterol, phospholipids, and liver sphingomyelin levels. These results indicated that both Sptlc1 and Sptlc2 interactions are necessary for SPT activity in vivo, and that SPT activity directly influences plasma sphingolipid levels. Furthermore, manipulation of SPT activity might well influence the course of such diseases as atherosclerosis.

1. Introduction

Serine palmitoyl-CoA transferase (SPT) is the rate-limiting enzyme in the biosynthesis of sphingolipids [1]. It has long been known that it plays an important role in the metabolism of sphingolipid. In addition, SPT activity in rat liver [2] and lung [3] is positively related to sphingolipid formation in those tissues. The activity of SPT is heightened in the aortas of rabbits fed a high cholesterol diet [4].

Two candidate cDNAs for yeast SPT, termed LCB1 and LCB2, have been cloned [5,6], and the translated sequences indicate that their gene products have a 21% amino acid sequence identity [6]. The lack of SPT activity in a yeast strain defective in LCB1 or LCB2, together with the protein similarity data, suggest that the two genes encode subunits of SPT [6]. Mouse and human LCB1 and LCB2 cDNA homologues have also been cloned [7,8]. In mouse, the two mRNAs have the same tissue distribution, and the ratio of the two transcript amounts remains approximately constant in all tissues [8]. The tissue distribution of Sptlc2 mRNA parallels the distribution of SPT activity [9].

It has been shown that mammalian SPT is a heterodimer of 53-kDa Sptlc1 and 63-kDa Sptlc2 subunits [8,10], both of which are bound to the endoplasmic reticulum (ER) [11]. Sptlc2 appears to be unstable unless it is associated with Sptlc1 [11]. SPT activity can be regulated transcriptionally and post-transcriptionally, and its up-regulation has been suggested as playing a role in the apoptosis induced by certain types of stress [12]. Specific missense mutations in the human LCB1

Abbreviations: SPT, serine palmitoyl-CoA transferase; LCB, long chain base; Cer, ceramide; Sph, sphingosine; S1P, sphingosine-1-phosphate; SM, sphingomyelin; PL, choline-containing phospholipids; TG, triglyceride; FPLC, fast protein liquid chromatography

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gene cause hereditary sensory neuropathy type I, an autosomal dominant, inherited disease, and these mutations confer dominant-negative effects on SPT activity [13,14].

In this study, we applied homologous recombination to embryonic stem cells, producing mice with an Sptlc1 or Sptlc2 gene deficiency to evaluate the in vivo role of SPT in sphingolipid metabolism, as well as the relationship between Sptlc1 and Sptlc2. We found that both Sptlc1 and Sptlc2 are responsible for SPT activity, that homozygous deficiency of Sptlc1 or Sptlc2 caused embryonic death, and that a heterozygous deficiency of the Sptlc1 or Sptlc2 gene causes significant changes of plasma sphingolipids, including ceramide (Cer) and sphingosine-1-phosphate (S1P) levels.

2. Experimental procedures

2.1. Construction of gene replacement vector for Sptlc1

A 12 kb mouse genomic DNA fragment, containing Sptlc1 exons 7–10 from the mouse 129 lambda genomic library, was utilized for targeting vector construction (Fig. 1). Embryonic stem (ES) cells were electroporated by *Pac*I-linearized targeting vector, and screened by selection with G418. Southern blot analysis and PCR were used for screening the targeted ES cells. Genomic DNA was digested with *ECo*RV and a 350-bp DNA fragment, just 3′ to the targeting vector, (Fig. 2), was used as a probe for Southern blots.

The WT contained a 7.2-kb fragment, while the recombinant contained a 5.5 kb fragment without exon 7 or 8 (Fig. 1B). PCR was done using primer pairs SrSA5 and Neo2. Primer SrSA5 was located outside the short arm, with a sequence of 5′-TCAGAGATTCTCCATTGCCACTG-3′. Primer Neo2 was

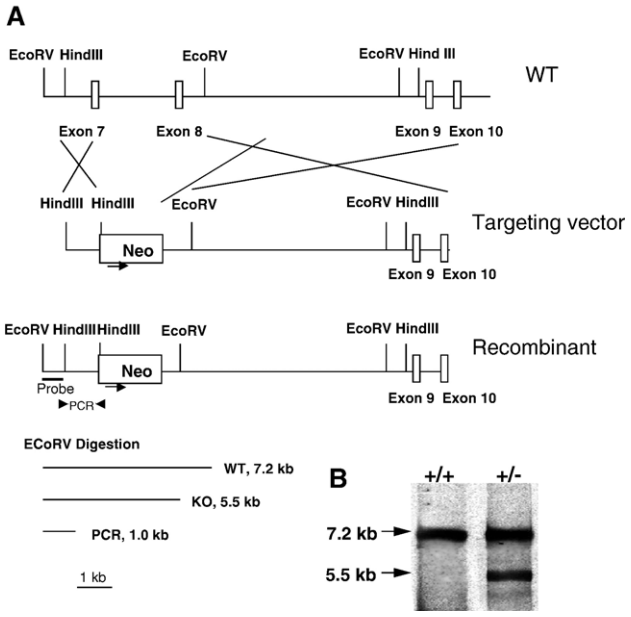

Fig. 1. Strategy used to disrupt the mouse LCB1 gene. (a) The top line represents the map of the endogenous murine LCB1 gene and its flanking sequence. The middle line represents the vector used to target the LCB1 locus. The bottom line shows the predicted organization of the locus after homologous recombination. A probe and a pair of PCR primers indicated in this line were used to confirm the integrity of site-specific integration. (b) Southern blot analysis of mouse tail-tip genomic DNA digested with *Eco*RV and hybridized with the probe. WT mouse DNA has a 7.2-kb signal only (+/+); heterozygous deficient mouse DNA has both a 7.2-kb and a 5.5-kb signal (+/−). Neo, neomycin-resistant gene. The arrow in Neo cassette indicates the direction of the gene transcription.

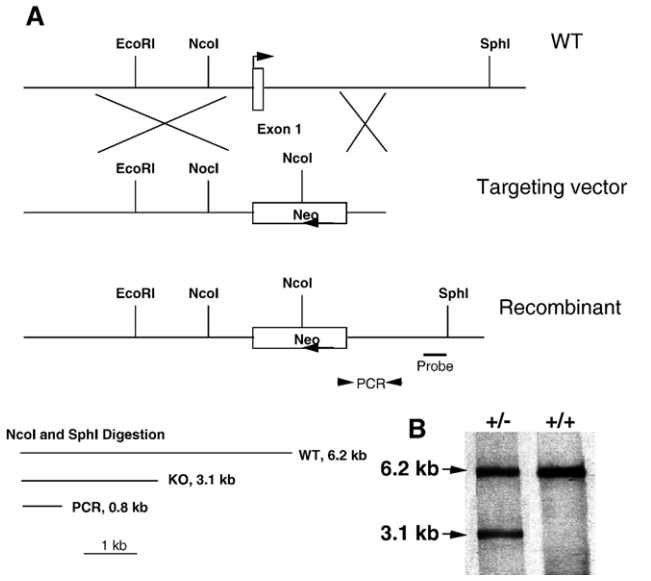

Fig. 2. Strategy used to disrupt the mouse LCB2 gene. (a) The top line represents the map of the endogenous murine LCB2 gene and its flanking sequence. The middle line represents the vector used to target the LCB2 locus. The bottom line shows the predicted organization of the locus after homologous recombination. A probe and a pair of PCR primers indicated in this line were used to confirm the integrity of site-specific integration. (b) Southern blot analysis of mouse tail-tip genomic DNA digested with *Nco*I/*Sph*I and hybridized with the probe. WT mouse DNA has a 6.2-kb signal only (+/+); heterozygous deficient mouse DNA has both a 6.2-kb and a 3.1-kb signal (+/−). Neo, neomycin-resistant gene. The arrow in Neo cassette indicates the direction of the gene transcription.

located in the 5′-promoter region of the neo gene cassette, with a sequence of 5′-TGCTGTCCATCTGCACGAGA-3′. The positive clones gave rise to a 1.0-kb PCR fragment. The correctly targeted ES cell lines were microinjected into C57BL/6J blastocysts. Chimeric mice were generated, and provided germline transmission of the disrupted Sptlc1 gene.

2.2. Construction of gene replacement vector for Sptlc2

The overall strategy for Sptlc2 gene targeting was to replace exon 1 with the neomycin-resistant gene (Fig. 2). Because exon 1 contains the translation initiation codon ATG, deletion of exon 1 would be expected to create a null Sptlc2 mouse model. We cloned a genetic fragment of Sptlc2 by screening a mouse genomic library. This clone contained 7.5 kb of 5′ flanking region exon 1, and 4.5 kb of intron 1 of the mouse Sptlc2 gene, and was used for gene targeting vector construction (Fig. 2). ES cells were electroporated by *Pac*I-linearized targeting vector, and screened by selection with G418. Southern blot analysis and PCR were used for screening the targeted ES cells.

Genomic DNA was digested with *Nco*I and *Sph*I, and a 300-bp DNA fragment, just 3′ to the targeting vector (Fig. 2), was used as a probe for Southern blots. The WT contained a 6.2-kb fragment, while the recombinant contained a 3.1-kb fragment without exon 1 (Fig. 2B). Two primers (SPTSA1 and Neo1), one located outside of the targeting vector with a sequence of 5′-CAGGACTCATGACAACTTACC-3′ and the other at the 5′ end of the neomycin-resistant gene with a sequence of 5′-TGCGAGGCCAGAGGCCACTTGTGTAGC-3′ (Fig. 2), were used to perform PCR. The positive clones gave rise to a 0.8-kb PCR fragment. The correctly targeted ES cell lines were microinjected into C57BL/6J blastocysts. Chimeric mice were generated, and provided germline transmission of the disrupted Sptlc2 gene.

2.3. Animals and diets used in this study

Chimeric males were mated with C57BL/6 females, and the resulting F1 animals containing the disrupted allele were intercrossed to generate F2 mice.