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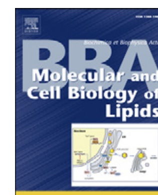

Disruption of *Stard10* gene alters the PPARα-mediated bile acid homeostasis

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ABSTRACT

STARD10, a member of the steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) protein family, is highly expressed in the liver and has been shown to transfer phosphatidylcholine. Therefore it has been assumed that STARD10 may function in the secretion of phospholipids into the bile. To help elucidate the physiological role of STARD10, we produced *Stard10* knockout mice (*Stard10*$^{-/-}$) and studied their phenotype. Neither liver content nor biliary secretion of phosphatidylcholine was altered in *Stard10*$^{-/-}$ mice. Unexpectedly, the biliary secretion of bile acids from the liver and the level of taurine-conjugated bile acids in the bile were significantly higher in *Stard10*$^{-/-}$ mice than wild type (WT) mice. In contrast, the levels of the secondary bile acids were lower in the liver of *Stard10*$^{-/-}$ mice, suggesting that the enterohepatic cycling is impaired. STARD10 was also expressed in the gallbladder and small intestine where the expression level of apical sodium dependent bile acid transporter (ASBT) turned out to be markedly lower in *Stard10*$^{-/-}$ mice than in WT mice when measured under fed condition. Consistent with the above results, the fecal excretion of bile acids was significantly increased in *Stard10*$^{-/-}$ mice. Interestingly, PPARα-dependent genes responsible for the regulation of bile acid metabolism were down-regulated in the liver of *Stard10*$^{-/-}$ mice. The loss of STARD10 impaired the PPARα activity and the expression of a PPARα-target gene such as *Cyp8b1* in mouse hepatoma cells. These results indicate that STARD10 is involved in regulating bile acid metabolism through the modulation of PPARα-mediated mechanism.

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1. Introduction

The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain containing 10 (STARD10) and STARD2 is a member of the START protein superfamily [1–3]. *In vitro* studies have shown that they transfer phospholipids such as phosphatidylcholine between membranes [4,5]. In mammals, more than 95% of biliary phospholipid consists of phosphatidylcholine [6]. Since STARD2 is highly expressed in the liver, it was assumed to be involved in regulating bile formation through the transport of biliary phosphatidylcholine to the canalicular membrane. This hypothesis, however, was disproved by studies on *Stard2* knockout mice [7,8]. Therefore other START family members expressed in the liver, such as *Stard10*, may be responsible for the transport of biliary phosphatidylcholine [2].

STARD10 was identified as a protein associated with the maturation of sperm [3]. STARD10 has been also linked with colon cancer [9] and breast

Abbreviations: StAR, steroidogenic acute regulatory protein; START, StAR-related lipid transfer; STARD10/*Stard10*, START domain containing 10; WT, wild type; ASBT/*Asbt*, apical sodium dependent bile acid transporter; STARD2/*Stard2*, START domain containing 2; CA, cholic acid; CDCA, chenodeoxycholic acid; CYP8B1/*Cyp8b1*, sterol 12α-hydroxylase; PPAR, peroxisome proliferator activated receptor; *Acot*, acyl-CoA thioesterase; *Acox1*, acyl-Coenzyme A oxidase 1; *Cpt1a*, carnitine palmitoyltransferase 1a; *Cyp4a10*, cytochrome P450 family 4 subfamily a polypeptide 10; FXR/*Fxr*, farnesoid X receptor; *Cyp7a1*, cholesterol 7α-hydroxylase; *Cyp7b1*, oxysterol 7α-hydroxylase; *Cyp27a1*, sterol 27-hydroxylase; BSEP/*Bsep*, bile salt export pump; MTP/*Mtp*, microsomal triglyceride transfer protein; HMGCR/*Hmgcr*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; *Hprt*, hypoxanthine-guanine phosphoribosyltransferase; RQ, relative quantity; MCA, α-muricholic acid; UDCA, ursodeoxycholic acid; HDCA, hyodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; TMCA, α-tauromuricholic acid; TCA, taurocholic acid; TUDCA, tauroursodeoxycholic acid; THDCA, taurohyodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid; GCA, glycocholic acid; GUDCA, glycoursodeoxycholic acid; GHDCA, glycohyodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid

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cancer [10], and recent studies have shown that the down-regulation of STARD10 by miR-611 is associated with the epithelial mesenchyme transition of breast cancer cells [11]. STARD10 has been also shown to be highly expressed in the liver [3]. However, the physiological role of STARD10 has been unknown.

To address the role of STARD10 in bile formation, we examined the impact of its genetic ablation in mice. We found that STARD10 is involved in regulating bile acid metabolism not through the regulation of phosphatidylcholine but through the PPARα-mediated mechanism.

2. Experimental procedures

2.1. Animals

The present study was approved by the Animal Care and Use Committee of Toho University and conducted according to the Law for Prevention of Cruelty to Animals and the Guide for Care and Use of Laboratory Animals in Japan. Mice were maintained in a 12:12 hour light–dark cycle at a regulated temperature of 23 ± 1 °C and humidity of 50 ± 10%. Mice were provided with food and water *ad libitum*. Normal diet (ND, CE-2) containing 4.2% fat and 54.6% carbohydrate or high-fat diet (HFD, HFD32) containing 32.0% fat and 29.4% carbohydrate were purchased from CLEA Japan (Tokyo, Japan).

2.2. Generation of Stard10 knockout mice

Stard10-deficient mice were produced by standard gene-targeting methods (see the Supplemental information). *Stard10*$^{+/-}$ mice were back-crossed with C57BL/6J mice for 9–14 generations.

2.3. Measurement of cholesterol, triglyceride, and serum fatty acid and alanine aminotransferase (ALT)

Livers were collected from mice and immediately frozen in liquid nitrogen. Lipids were extracted according to the method of Folch et al. [12]. Total liver cholesterol and triglyceride levels were determined using an enzymatic method with Cholestest CHO and Cholestest TG (Sekisui medical, Tokyo, Japan).

The serum fatty acid concentration was determined using fatty acid assay kit (BioVision, Milpitas, CA, USA). The fluorescence at Ex/Em = 535/595 nm was measured using a TriStar LB 940 spectrophotometer (Berthold, Bad Wildbad, Germany). The serum ALT level was determined with GPT/ALT-PIII kit (Fujifilm, Tokyo, Japan).

2.4. Measurement of ionic metabolite levels using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) system

Sample preparation, instrumentation, conditions, and data processing were carried out as described previously [13]. Livers from three different mice in each group were combined for normalization. The metabolic pathway map was produced using public-domain software [14].

2.5. Measurement of bile acids

Bile was collected from gallbladder under anesthesia. In some experiments, bile duct was cannulated under anesthesia as previously described [15], and the bile was collected for 3 h. Feces were collected for 48 h, and bile acids were extracted according to the method of Beher et al. [16].

Bile acid was measured using Total Bile Acids kit (Diazyme, Poway, CA, USA). Biliary components of bile acids were analyzed using UPLC–MS systems on an ACQUITY UPLC® Quattro Premier XE mass spectrometer (Waters, Milford, MA, USA) as previously described [15,17]. Phosphatidylcholine in the bile was measured using phospholipid C-test Wako (Wako, Osaka, Japan), and cholesterol with Cholesterol Quantitation Kit (BioVision).

2.6. Western blotting

Tissues were excised, immediately frozen in liquid nitrogen, then homogenized in ice-cold buffer and centrifuged at 1500 *g* for 10 min at 4 °C. The protein concentration was determined using the Coomassie Plus Better Bradford™ Assay Reagent (Pierce, Rockford, IL, USA). Samples were subjected to SDS-PAGE and proteins were transferred onto polyvinylidene difluoride membranes (Hybond-P; GE Healthcare, Piscataway, NJ, USA). Blots were probed with anti-STARD10 antibody (1/1000), anti-ASBT antibody (1/1000), antibody against bile salt export pump (BSEP) (1/1000), anti-CYP8B1 antibody (1/4000), anti-PMP70 antibody (1/5000), anti-MTP antibody (1/1000), anti-HMGCR (1/1000), anti-PPARα antibody (1/1000), or anti-α-tubulin antibody (1/5000), followed by horseradish peroxidase-conjugated secondary antibodies. Blots were visualized using an enhanced chemiluminescence system (GE Healthcare) and recorded with a LAS-1000 Image Analyzer (Fuji Film, Tokyo, Japan).

2.7. Immunohistochemistry

Cryostat sections (20 μm) were placed on Matsunami adhesive silane (MAS)-coated glass slides (Matsunami, Osaka, Japan) and incubated with primary antibodies overnight at 4 °C. Alexa-conjugated secondary antibodies were used for multi-color imaging (Life Technologies, Carlsbad, CA, USA). Hoechst 33258 (Life Technologies) was used to stain nuclei. Samples were analyzed on an LSM510-META laser-scanning confocal microscope (Carl-Zeiss, Göttingen, Germany).

2.8. Antibodies

A rabbit polyclonal antibody against mouse STARD10 protein was raised by immunizing rabbits with synthetic oligo-peptides (NH2-CIKYPEWKQKHQPHF-COOH and NH2-C + GAGGEGSDDDTSLT-COOH) by a custom antibody production service (Operon, Tokyo Japan). Antibodies against α-tubulin (T5168), cadherin (C1821), and PMP70 (SAB4200181) were purchased from Sigma (Saint Louis, MO, USA). Antibody against MTP (612022) was purchased from BD Biosciences (San Jose, CA, USA). Antibody against HMGCR (07-457) was purchased from Millipore (Temecula, CA, USA).

Antibody against PPARα (ab8934) was purchased from Abcam (Cambridge, MA, USA). Antibodies against CYP8B1 (sc23515) and ASBT (sc27494) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against BSEP was a generous gift from Dr. Kousei Ito of the University of Tokyo.

2.9. Luciferase reporter assay of PPARα activity

The knockdown of *Stard10* was performed with small interference RNA (siRNA) with oligonucleotides (5′-CCAAGUGGGUGGUGAAUAA TT-3′ and 5′-UUAUUCACCACCCACUUGGTT-3′). As a negative control, annealed double strand RNA oligonucleotides that have no target (S5C-0600, Cosmo bio, Tokyo, Japan) were used. For the overexpression of *Stard10*, cells were transfected with pcDNA4/HisMax-Stard10. Control experiments were performed with pcDNA4/HisMax-lacZ (K864-20, Life Technologies).

PPRE-X3-TK-luc contains three copies of PPAR response elements in the upstream of thymidine kinase promoter [18]. Hepa1–6 cells (CRL-1830; ATCC, Bethesda, MD, USA), mouse hepatoma cell line, were transfected with PPRE X3-TK-luc (1015: addgene, Cambridge, MA, USA), pGL4.74-hRluc/TK (E6921: Promega, Madison, WI, USA), and either siRNA or Strad10 expression plasmid using lipofectamine2000

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