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Intestine-Specific Deletion of SIRT1 in Mice Impairs DCoH2–HNF-1α–FXR Signaling and Alters Systemic Bile Acid Homeostasis

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BACKGROUND & AIMS: Sirtuin 1 (SIRT1), the most conserved mammalian oxidized nicotinamide adenine dinucleotide–dependent protein deacetylase, is an important metabolic sensor in many tissues. However, little is known about its role in the small intestine, which absorbs and senses nutrients. We investigated the functions of intestinal SIRT1 in systemic bile acid and cholesterol metabolism in mice. **METHODS:** SIRT1 was specifically deleted from the intestines of mice using the flox-Villin-Cre system (SIRT1 iKO mice). Intestinal and hepatic tissues were collected, and bile acid absorption was analyzed using the everted gut sac experiment. Systemic bile acid metabolism was studied in SIRT1 iKO and flox control mice placed on standard diets, diets containing 0.5% cholic acid or 1.25% cholesterol, or lithogenic diets. **RESULTS:** SIRT1 iKO mice had reduced intestinal farnesoid X receptor (FXR) signaling via hepatocyte nuclear factor 1α (HNF-1α) compared with controls, which reduced expression of the bile acid transporter genes *Asbt* and *Mcf2l* (encodes Ost) and absorption of ileal bile acids. SIRT1 regulated HNF-1α/FXR signaling partially through dimerization cofactor of HNF-1a (Dcoh2) Dcoh2, which increases dimerization of HNF-1α. SIRT1 was found to deacetylate Dcoh2, promoting its interaction with HNF-1α and inducing DNA binding by HNF-1α. Intestine-specific deletion of SIRT1 increased hepatic bile acid biosynthesis, reduced hepatic accumulation of bile acids, and protected animals from liver damage from a diet high in levels of bile acids. **CONCLUSIONS:** Intestinal SIRT1, a key nutrient sensor, is required for ileal bile acid absorption and systemic bile acid homeostasis in mice. We delineated the mechanism of metabolic regulation of HNF-1α/FXR signaling. Reagents designed to inhibit intestinal SIRT1 might be developed to treat bile acid–related diseases such as cholestasis.

Keywords: Ileal Bile Acid Absorption; Bile Acid Synthesis; Liver Damage; Cholestasis.

Bile acids are the major end products of hepatic cholesterol catabolism. They are essential for intestinal absorption of dietary lipids, cholesterol, and fat-soluble vitamins and play an important role in cholesterol metabolism. Whole body bile acid homeostasis is maintained by efficient enterohepatic cycling of bile acids between the liver and small intestine, in which 95% of bile acids released from the liver into the proximal duodenum in response to entering of dietary fats are reabsorbed by the distal ileum and transported back to the liver via the portal circulation.[1–3] The unabsorbed 5% of the bile acids eliminated through feces in each enterohepatic cycle will then be supplemented by new hepatic synthesis so that a constant pool of bile acids is maintained. In addition, a small amount of bile acids that spill over into the systemic circulation will be excreted in urine. Therefore, bile acid metabolism is tightly regulated by various nutritional and hormonal cues, and its dysregulation has been associated with a number of gastrointestinal and metabolic diseases, including cholestasis, hypercholesterolemia, defective liver regeneration, cholesterol gallstone disease, and diabetes.[4–7]

A key regulatory factor for systemic bile acid metabolism is farnesoid X receptor (FXR), a cellular bile acid sensor and a member of adopted orphan nuclear receptors.[2,8,9] In enterocytes, activation of FXR by bile acids enhances the transcription of a number of bile acid transporters, particularly the intestinal basolateral organic solute transporters Ostα/Ostβ, which are responsible for bile acid export from enterocytes into portal blood.[10] Activation of FXR in enterocytes also induces a hormone, fibroblast growth factor 15 (FGF15), which travels to the liver and represses hepatic bile acid biosynthesis through its receptor, Fgfr4, and the c-Jun-N-terminal kinase pathway.[2,11] In addition to FXR, hepatocyte nuclear factor 1α (HNF-1α), a homeodomain-containing transcription factor, is critical in the regulation of intestinal and systemic bile acid metabolism.[12] HNF-1α directly binds to the

Abbreviations used in this paper: **CA, cholic acid; DCoH2, dimerization cofactor of hepatocyte nuclear factor 1α 2; FGF15, fibroblast growth factor 15; FXR, farnesoid X receptor; HA, hemagglutinin; HNF-1α, hepatocyte nuclear factor 1α; iKO, intestinal-specific knockout; LD, lithogenic diet; SIRT1, sirtuin 1; WT, wild-type.**

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promoter of the FXR gene to modulate its expression.[12] HNF-1α also regulates ileal bile acid uptake through transcriptional induction of the apical sodium-dependent bile acid transporter Asbt.[13] Therefore, the intestinal HNF-1α and FXR signaling pathways tightly control ileal bile acid uptake and systemic bile acid homeostasis in response to nutritional and hormonal signals.

Sirtuin 1 (SIRT1) is a member of sirtuins, a family of highly conserved oxidized nicotinamide adenine dinucleotide–dependent protein deacetylases and/or adenosine diphosphate ribosyltransferases.[14] Accumulating evidence has indicated that sirtuins are crucial regulators for a variety of cellular processes, ranging from energy metabolism and stress response to tumorigenesis and aging.[15,16] As the most conserved mammalian sirtuin, SIRT1 couples the deacetylation of numerous transcription factors and cofactors to the cleavage of oxidized nicotinamide adenine dinucleotide, an indicator of cellular metabolic status.[17,18] Therefore, SIRT1 is an important regulator of energy homeostasis in several tissues, including the liver, adipose tissues, pancreas, and hypothalamus.[18,19] However, the function of SIRT1 in the small intestine, particularly in intestinal nutrient absorption, has not yet been determined.

To investigate the role of SIRT1 in intestinal physiology, we generated an intestine-specific SIRT1 knockout mouse strain (SIRT1 iKO). In this report, we show that intestinal SIRT1 regulates ileal bile acid absorption and that feedback affects systemic bile acid and cholesterol metabolism in mice. Intestinal SIRT1 modulates the DNA binding ability of HNF-1α, partially through deacetylation of a dimerization cofactor of HNF-1α, pterin 4α carbinolamine dehydratase 2/dimerization cofactor of HNF-1α 2 (DCoH2), which is a novel acetylated protein. Consequently, deletion of intestinal SIRT1 decreases the expression of FXR and Asbt, reducing ileal bile acid absorption. Intriguingly, in contrast to hepatic deficiency of SIRT1, which leads to decreased HNF-1α/FXR signaling pathways, diminished hepatic bile acid excretion, and increased liver damage,[20] deletion of intestinal SIRT1 reduces hepatic accumulation of bile acids and blunts the inhibition of hepatic bile acid synthesis in diets high in levels of bile acids, protecting animals from liver damage.

Materials and Methods

Animal Experiments

The SIRT1 iKO mice in a C57BL/6 background were generated by crossing mice carrying a SIRT1 exon 4 floxed allele[21] with Villin-Cre mice (Jackson Laboratory Ben Harbor, ME.[22]). Three- to four-month old SIRT1 iKO mice (Villin-Cre^{+}, SIRT1$^{flox/flox}$) and their littermate flox controls (Villin-Cre^{-}, SIRT1$^{flox/flox}$) as well as age-matched wild-type (WT) and Villin-Cre mice were fed a standard laboratory chow diet, a chow diet containing 0.5% cholic acid (CA) (custom made; Research Diets, New Brunswick, NJ), a chow diet containing 1.25% cholesterol (custom made; Research Diets), or a lithogenic diet (LD) (D12383; Research Diets) for indicated times. All animal experiments were approved by the National Institute of Environmental Health Sciences/National Institutes of Health Animal Care and Use Committee.

Analysis of Bile Acids

To determine the output of fecal bile acids, feces were collected from individually housed mice over 48 hours and fecal bile acids were extracted with 75% ethanol at 50°C for 2 hours. To determine the total bile acid pool size, liver, gallbladder, and full-length small intestine were dissected and homogenized in water and extracted with 75% ethanol. Bile acids in the resulting supernatants were measured using a total bile acid kit (Diazyme Laboratories, Poway, CA). Gallbladder bile acid profiles were analyzed using ultra-performance liquid chromatography/tandem mass spectrometry as described.[23]

Everted Gut Sac Experiment

The everted gut sac experiments were performed essentially as described.[10]

Chromatin Immunoprecipitation Analysis

Chromatin immunoprecipitation analysis was performed essentially as described by the manufacturer (Millipore, Billerica, MA) with some modifications. Briefly, flox and SIRT1 iKO mice were perfused with 1% paraformaldehyde in phosphate-buffered saline at room temperature via heart perfusion to cross-link protein-DNA complexes. After 10 minutes, the cross-linking was stopped by perfusion with 0.125 mol/L glycine. The sonicated cross-linked chromatin was subjected to immunoprecipition with antibodies against FXR, HNF-1α (Santa Cruz Biotechnology, Santa Cruz, CA), or normal rabbit immunoglobulin G. DNA fragments were analyzed by quantitative polymerase chain reaction using primers flanking indicated promoter regions.

Protein Acetylation Analysis

To analyze the deacetylation of DCoH2 by SIRT1, HEK293T SIRT1 RNA interference cells were transfected with constructs expressing HA-DCoH2, with or without FLAG-p300 and SIRT1 as indicated. Forty-eight hours after transfection, cells were treated with 5 μmol/L Trichostatin A for 2 hours, harvested, and homogenized. HA-DCoH2 was immunopurified with anti-HA beads (Santa Cruz Biotechnology), subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and analyzed with anti–acetyl lysine polyclonal antibodies (Cell Signaling Technology, Danvers, MA).

Identification of Lysine Acetylation Sites in DCoH2 Protein

The 2 acetylated sites (K124 and K131) in DCoH2 (Supplementary Figure 4) were identified by proteomics screening as described.[24,25]

Statistical Analysis

Values are expressed as mean ± SEM. Significant differences between the means were analyzed by the 2-tailed, unpaired, nonparametric Mann–Whitney test, and differences were considered significant at $P < .05$.