Bile acid represses the peroxisome proliferator-activated receptor-γ coactivator-1 promoter activity in a small heterodimer partner-dependent manner

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Abstract. Bile acid homeostasis is tightly controlled by the feedback mechanism in which an atypical orphan nuclear receptor (NR), small heterodimer partner (SHP), inactivates several transcription factors. We previously demonstrated that bile acid represses the expression of gluconeogenic genes, including glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), and fructose-1,6-bisphosphatase (FBP1) in an SHP-dependent manner. Recently, peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1 (PGC-1) gene, a coactivator of NRs important for gluconeogenic gene expression, was also downregulated by bile acid in wild-type mice but not in farnesoid X receptor- or SHP-null mice. However, the molecular mechanism for the effect of bile acid on PGC-1 gene expression remains unknown. In the present study, a series of reporter assays demonstrated that the promoter activity of PGC-1 via a member of the forkhead transcription factors, Foxo1, FOXO3a, and Foxo4 was downregulated by treatment with chenodeoxicholic acid and with transfected SHP. These results revealed that bile acid inhibits the promoter activity of PGC-1 in an SHP-dependent manner.

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Abbreviations: NR, nuclear receptor; FXR, farnesoid X receptor; CDCA, chenodeoxycholic acid; CYP7A1, cholesterol 7α-hydroxylase; LRH-1, liver receptor homolog-1; SHP, small heterodimer partner; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; FBP1, fructose-1,6-bisphosphatase; CREB, cAMP response element binding protein; PGC-1, peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1; BAT, brown adipocytes; ERRγ, estrogen-related receptor γ; GPCR, G-protein-coupled receptor

Key words: bile acid, farnesoid X receptor, peroxisome proliferators-activated receptor-γ (PPAR-γ) coactivator-1, small heterodimer partner

Introduction

Bile acid plays a critical role in the elimination of excess cholesterol in the liver and endogenous ligands for farnesoid X receptor (FXR), a member of the nuclear receptor (NR) superfamily that is primarily expressed in the liver, kidney and intestine (1). FXR acts as an important feedback regulator of bile acid biosynthesis via transcriptional repression of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme of bile acid biosynthesis, by inducing expression of the orphan nuclear receptor small heterodimer partner (SHP) (2,3). Bile acid is also important for glucose homeostasis (4-7). We and others indicated that bile acid alters expression of the genes involved in gluconeogenesis, including glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), and fructose-1,6-bisphosphatase (FBP1) (4,5,8). A recent study demonstrated that FXR-null mice had elevated serum glucose and developed insulin resistance and that FXR was essential for normal glucose homeostasis *in vivo* (8).

Peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1 (PGC-1), a nuclear receptor coactivator that interacts with PPAR-γ (9), functions to link nuclear receptors to the transcriptional program of gluconeogenesis (10,11). PGC-1 is strongly induced in the liver in fasting mice and in three mouse models deficient for insulin action (10). cAMP response element binding protein (CREB) binds to the target sequence [cAMP response element (CRE)] in the PGC-1 promoter, thereby enhancing the expression of PGC-1 in response to cAMP (11). In contrast, we showed that Foxo1 activates the PGC-1 promoter via insulin response sequences (IRSs) and suppresses its activity through insulin stimulation (12). In addition to insulin, it has been demonstrated that bile acid represses not only gluconeogenic genes but also the PGC-1 gene in wild-type mice but neither FXR- nor SHP-null mice (8). These observations indicated that bile acid decreases the expression of the PGC-1 gene in an FXR- and SHP-dependent manner. However, the molecular mechanism of these responses remains unclear.

Here, we showed that bile acid and transfected SHP directly repressed the PGC-1 promoter activity in HepG2 cells. We found that both bile acid and SHP counteract the induced PGC-1 promoter activity by the FoxO family. Bile acid and transfected SHP suppressed Foxo1-mediated PGC-1

promoter activity in a PKB-independent manner. These results suggest that SHP-mediated Foxo1 transinactivation in the PGC-1 gene confers the suppressive effect of bile acid on PGC-1 expression.

Materials and methods

Cloning and plasmids. The mouse Foxo1 and Foxo4 cDNAs were subcloned into pcDNA3 tagged with the FLAG epitope (13,14). The human FOXO3a cDNA was cloned by reverse transcription-PCR and subcloned into the *Bam*HI site in the pcDNA3-FLAG vector. Foxo1 S253A and 3A mutants, the latter of which is mutated at the three PKB phosphorylation sites (T24, S253, and S316) to alanines, were generated by PCR-based mutagenesis (15). The PGC-1-luc and pcDNA3-HA-SHP plasmids have been described previously (5,12,16).

Cell culture, transfections, and reporter gene assays. HepG2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and transfections were performed by GeneJuice reagents (Novagen). pCMV-ß-galactosidase plasmid was included in each transfection experiment to control for the efficiency of transfection. To ensure equal DNA amounts, empty plasmids were added in each transfection. Five hours after transfection, medium was replaced with serum-free DMEM in the presence or absence of chenodeoxycholic acid (CDCA, Sigma) in the indicated concentrations, and cells were further incubated for 24 h. The luciferase activity was measured with Wallac (PerkinElmer) and normalized for ß-galactosidase activity in the same sample.

Results

Bile acid represses PGC-1 promoter activity via an SHP-dependent manner. Previously, we and others observed that bile acid represses hepatic gluconeogenic and its related gene expression, i.e. G6Pase, PEPCK, FBP1, and PGC-1 genes in C57BL/6J mice (4,5,8,17). Furthermore, we showed that bile acid-dependent repression of gluconeogenic genes was due to an SHP-mediated pathway (5).

To elucidate the mechanism by which cholic acid down-regulates the PGC-1 gene expression, we performed reporter assays using human PGC-1 promoter-luciferase construct (12). HepG2 cells were transfected with each reporter construct and then treated with increasing concentrations of chenodeoxycholic acid (CDCA), a primary bile acid which induces SHP via the FXR-dependent pathway (2,3,18,19). As expected from the results that bile acid repressed the PGC-1 gene in mice (8,17), CDCA led to a dose-dependent decrease in the reporter gene activity (Fig. 1A). We also confirmed that CDCA repressed the G6Pase promoter activity (data not shown). These data indicate that bile acid also repressed PGC-1 gene expression at the transcriptional level.

It has been shown that bile acid induces SHP protein via the FXR-dependent pathway (2,3,18,19). SHP dimerizes with a subset of nuclear receptors or transcription factors including Foxo1, and represses their transactivation activities (20,21). To assess whether CDCA represses promoter activity of the PGC-1 gene through the FXR/SHP-dependent pathway, we examined the effects of SHP on the PGC-1 promoters. To this end, increasing amounts of SHP expression plasmids were cotransfected into HepG2 cells together with the PGC-1 promoter reporter constructs. As shown in Fig. 1B, SHP significantly repressed not only the G6Pase promoter (5) (data not shown), but also the PGC-1 promoter in a dose-dependent manner. These findings suggest that bile acid represses PGC-1 promoter activity via an SHP-dependent manner in HepG2 cells.

Bile acid decreases FoxO family-mediated activation of PGC-1 promoter via an SHP-dependent manner. Next, we sought to address how CDCA represses the PGC-1 promoter activity in HepG2 cells. The PGC-1 gene is upregulated by

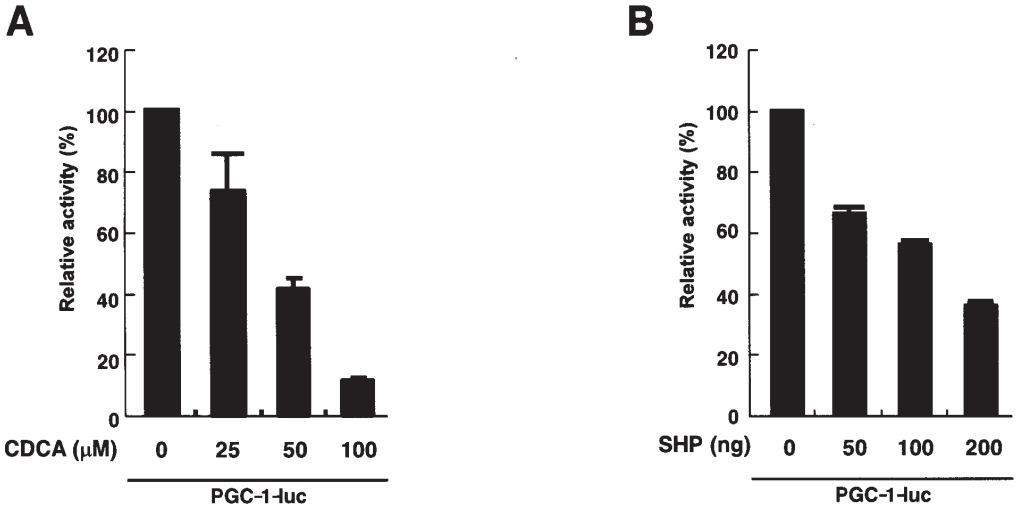

Figure 1. Downregulation of gluconeogenic gene expression by CDCA and SHP in HepG2 cells. (A) HepG2 cells were transfected with human PGC-1 promoter-luciferase plasmid. Following transfections, cells were serum-starved for 24 h in the presence or absence of increasing concentrations of CDCA (25, 50, and 100 μM). (B) HepG2 cells were transfected with PGC-1 promoter-luciferase constructs together with empty vectors or increasing amounts of SHP expression plasmid (50, 100, and 200 ng). The results are presented as relative luciferase activities compared with the activity in the absence of CDCA treatment (A) or SHP expression (B), which was set as 100%.

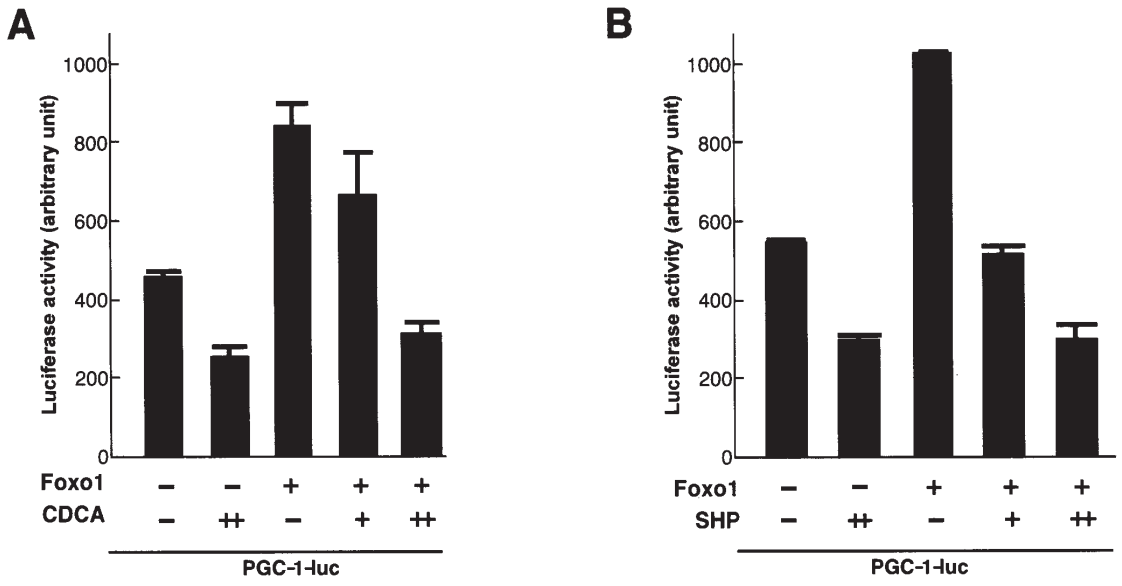

Figure 2. Repression of PGC-1 gene promoter activities by CDCA or SHP via Foxo1. (A) HepG2 cells were cotransfected with PGC-1 promoter-luciferase construct with or without 50 ng of Foxo1 expression plasmid. Following transfections, cells were serum-starved for 24 h in the presence or absence of increasing concentrations of CDCA (50 and 100 μM). (B) HepG2 cells were cotransfected with PGC-1 promoter-luciferase construct with or without either 50 ng of Foxo1 expression plasmid together with increasing amounts of SHP expression plasmid (50 and 200 ng). The results are presented as arbitrary units.

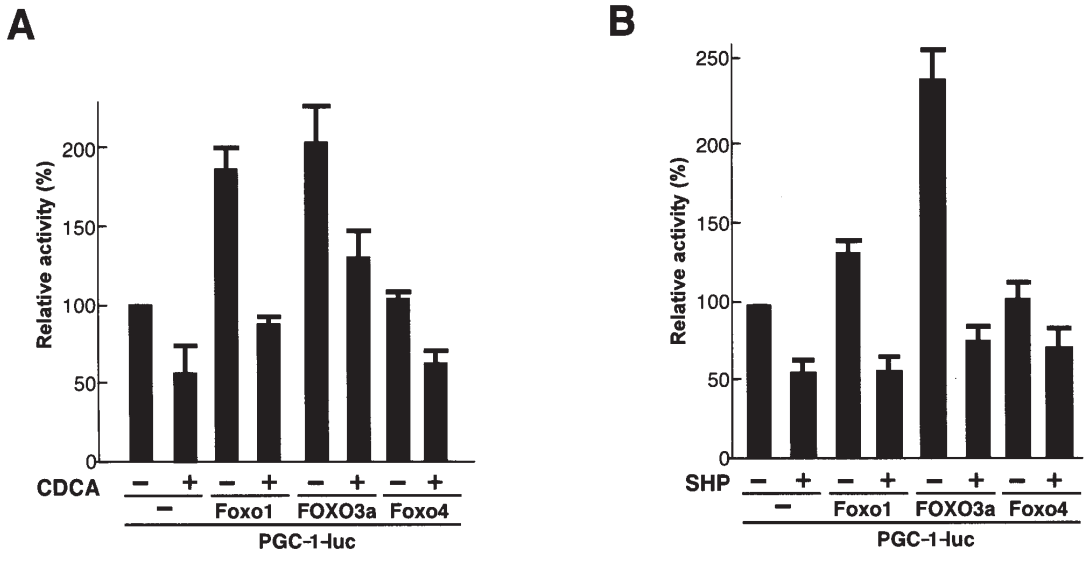

Figure 3. CDCA or SHP represses FoxO family-mediated PGC-1 promoter activities. (A) HepG2 cells were cotransfected with PGC-1 promoter-luciferase construct with or without 50 ng of Foxo1, FOXO3a, or Foxo4 expression plasmids. Following transfections, cells were serum-starved for 24 h in the presence or absence of 100 μM CDCA. (B) HepG2 cells were cotransfected with PGC-1 promoter-luciferase construct with or without either 50 ng of Foxo1, FOXO3a, or Foxo4 expression plasmids together with 200 ng of empty plasmid or SHP expression plasmid. The results are presented as relative luciferase activities compared with the activity in the absence of CDCA treatment (A) or SHP (B), which was set as 100%.

glucagons via the cAMP-PKA-CREB pathway (11) and is downregulated by insulin via the PI3K-PKB-Foxo1 pathway (12). Glucagon stimulates protein kinase A, which in turn phosphorylates the CREB protein at Ser-133, thereby promoting gluconeogenesis through the induction of PGC-1. However, enterohepatic circulation of bile acid occurs in the fed state when serum levels of glucagons and phosphorylation levels of hepatic CREB proteins are low but serum levels of insulin are high (22). Thus, we hypothesized that bile acid represses the PGC-1 promoter activities through Foxo1-mediated transcriptions.

To further clarify the repressive mechanism of CDCA, HepG2 cells were cotransfected with PGC-1 promoter-luciferase constructs together with Foxo1 expression plasmid following incubation with CDCA. In the absence of CDCA, Foxo1 stimulated the promoter activities of PGC-1, while CDCA drastically inhibited the Foxo1-mediated transactivation of the promoters (Fig. 2A). Taken together, these results suggest that CDCA downregulates the transactivation function of Foxo1 on the expression of the PGC-1 gene.

Since SHP represses the transactivation of Foxo1 at G6Pase promoter (5), we examined the effect of SHP on the

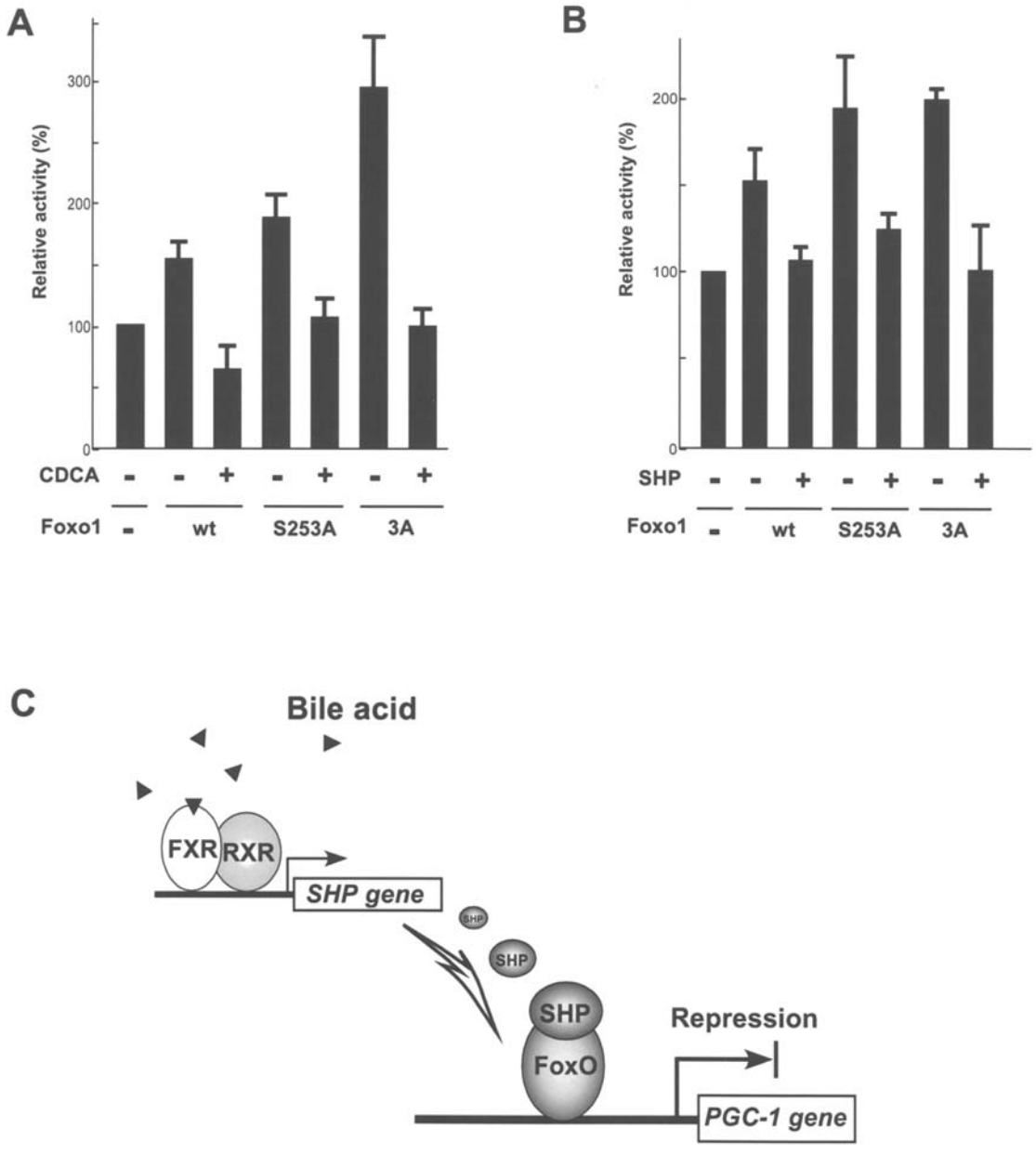

Figure 4. CDCA or SHP represses Foxo1-mediated PGC-1 promoter activities via a PKB-independent manner. (A) HepG2 cells were cotransfected with PGC-1 promoter-luciferase construct with or without 50 ng of Foxo1wt, S253A or 3A expression plasmids. Following transfections, cells were serum-starved for 24 h in the presence or absence of 100 μM CDCA. (B) HepG2 cells were cotransfected with PGC-1 promoter-luciferase construct with or without either 50 ng of Foxo1wt, S253A or 3A expression plasmids together with 100 ng of empty plasmid or SHP expression plasmid. The results are presented as relative luciferase activities compared with the activity in the absence of CDCA treatment (A) or SHP (B), which were set as 100%.

Foxo1-mediated PGC-1 transcription. As expected, SHP potently repressed Foxo1-stimulated PGC-1 promoter activity in a dose-dependent manner (Fig. 2B). In addition, both CDCA and SHP also repressed the FOXO3a- and Foxo4-mediated activation of PGC-1 promoter (Fig. 3A and B). These results suggest that bile acid downregulates the promoter activity of not only gluconeogenic gene expression but also PGC-1 gene expression in an SHP-dependent fashion via the FoxO family.

Bile acid decreases Foxo1-mediated activation of PGC-1 promoters via a PKB-independent manner. We previously demonstrated that insulin signaling via PKB to Foxo1 partly accounts for the effect of insulin to regulate the PGC-1 promoter activity via the IRSs (12). To investigate the effect of bile acid on the PGC-1 promoter via a PKB-dependent manner, we used Foxo1 S253A and 3A mutants, the latter of which is mutated at the three PKB phosphorylation sites (T24, S253, and S316) to alanines (15). These mutants diminished insulin-mediated suppression of transcriptional activities on the PGC-1 promoter (12) (data not shown). HepG2 cells were cotransfected with PGC-1 promoter-luciferase constructs together with various Foxo1 mutants following incubation with CDCA. As shown in Fig. 4A, CDCA drastically decreased both S253A and 3A mutants, suggesting that CDCA downregulates the promoter activity of the PGC-1 gene in a PKB-independent manner. In addition, SHP also suppressed these mutant-mediated PGC-1 promoter

activities (Fig. 4B). These results indicate that bile acid downregulates the promoter activity of the PGC-1 gene in a PKB-independent manner.

Discussion

In this study, we investigated the molecular mechanism for the effect of bile acid on PGC-1 gene expression. CDCA and transfected SHP directly downregulate basal (Fig. 1) or FoxO family-stimulated PGC-1 promoter activity in HepG2 cells (Figs. 2 and 3). CDCA and SHP but not insulin also decrease the PGC-1 promoter activity transactivated by Foxo1 mutants deficient for phosphorylation sites (Fig. 4), which indicates that bile acid represses PGC-1 expression via a PKB-independent manner. Based on these results, we summarized a model for bile acid-mediated downregulation of the PGC-1 gene via induced SHP (Fig. 4C). This supports our previous proposed model that bile acid represses gluconeogenic gene promoters in an SHP-dependent manner (5). Furthermore, our results also suggest that bile acid suppresses the PGC-1 gene via an insulin signal-independent manner (12).

Insulin is produced in the islets of Langerhans in the pancreas after feeding and rapidly decreases gluconeogenesis via the downregulation of G6Pase and PGC-1 genes (23,24). Moreover, we and others demonstrated that bile acid also represses the expression of both gluconeogenic enzyme(s) and PGC-1 (4,5,8,17). Compared with insulin, enterohepatic circulation of bile acid regulates their long-term homeostasis. Thus, bile acid may maintain gluconeogenic and related genes at basal expression for a long period after feeding.

Studies with FXR-null mice revealed that FXR is essential not only for its own homeostasis but also for normal glucose homeostasis (6,8,25-27). In contrast, mice lacking SHP show increased basal expression of PGC-1 in brown adipocytes (BAT) and increased energy expenditure (28). We argued that SHP represses the orphan nuclear receptor estrogen-related receptor γ (ERRγ-mediated transactivation of the PGC-1 promoter in BAT). In our present study, we provide a new line of evidence concerning Foxo1-mediated repression of PGC-1 promoter activity by SHP in HepG2 cells. These findings indicate that SHP has multiple target-proteins in various tissues and affects the expression of the PGC-1 gene.

Recent studies demonstrated that bile acid regulates multiple metabolic pathways including bile acid synthesis, conjugation, and transport, as well as various aspects of lipid and glucose metabolism (1). Activation of the nuclear receptor FXR by specific agonists improves hyperglycemia and hyperlipidemia in diabetic mice (29,30). Moreover, bile acid induces energy expenditure by promoting intracellular thyroid hormone activation via G-protein-coupled receptor (GPCR) TGR5 (17). Thus, bile acid receptors could be a pharmacological target for therapeutic applications, and screening their ligands will be one of the important approaches for drug development (31,32). Because PGC-1 is powerfully induced in the livers of diabetic model animals and is related to their pathology, our model that bile acid inhibits the promoter activity of PGC-1 in an SHP-dependent fashion will be a very important benchmark for drug discovery.

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