

Pharmacy Research at URI: Bile Acids and Bile Salt Export Pump: Physiology and Pathology

Ruitang Deng, PhD

THE LONG-TERM OBJECTIVE OF OUR RESEARCH program is to understand how bile acid homeostasis is regulated in physiological as well as pathological conditions, with a focus on the transcriptional regulation of the **bile salt export pump (BSEP)**.

As one of the major constituents of bile, bile acids were once considered bodily waste with no useful functions. Now it is well established that bile acids have important physiological functions in animals and human.¹ First, the well-known function of bile acids is to solubilize cholesterol and lipids in an aqueous environment, such as in the bile and the intestines. On the other hand, as biological detergents, too much bile acids are toxic for cells. Second, bile acids are recognized as signaling molecules serving as the endogenous ligands for nuclear receptor **farnesoid x receptor (FXR)**

and G-protein-coupled receptor **TGR5** to regulate cholesterol, bile acids and glucose homeostasis. Finally, recent studies have extended the functions of bile acids into various non-metabolic areas including inflammation, liver regeneration, hepatocarcinogenesis, inhibition of intestine bacterial growth and colon cancer.

Bile acid homeostasis is achieved through coordinately regulated bile acid synthesis and elimination pathways. **Cholesterol 7 α -hydroxylase (CYP7A1)** is the rate-limiting enzyme for bile acid synthesis while BSEP is the rate limiting-step in the enterohepatic circulation of bile acids.² (Figure 1) Modulation of BSEP expression or function by inherited or acquired factors has profound impacts on bile acid homeostasis and subsequently contributes to the risk for various bile

acids-associated diseases, including intra-hepatic cholestasis, gallstone disease and **hepatocellular carcinoma (HCC)**. Studying the transcriptional regulation of BSEP will uncover the mechanistic insights in the role BSEP plays in physiological as well as pathological conditions.

BSEP expression is regulated by nuclear receptor liver receptor homolog 1 (LRH-1) and oxysterols.

The expression of BSEP is positively regulated by the bile acid/FXR signaling pathway.³ Activation of FXR by bile acids strongly induces BSEP expression *in vitro* and *in vivo*. Such feed-forward regulation of BSEP by FXR is considered a major mechanism for preventing excessive accumulation of toxic bile acids in hepatocytes.

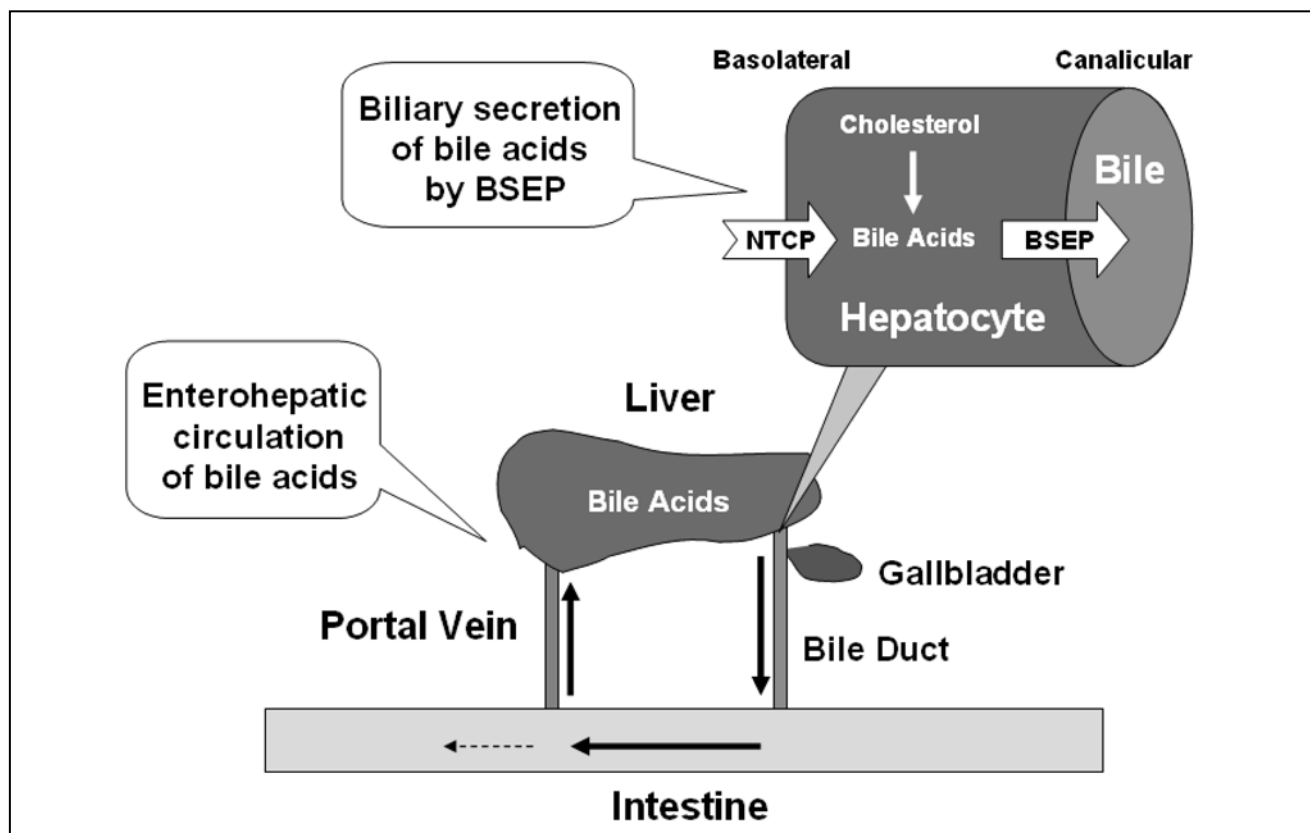


Figure 1. **Enterohepatic circulation of bile acids.** Bile acids are synthesized in the liver from cholesterol, secreted into the bile through BSEP, reabsorbed in the intestine, and return to the liver via the portal vein. In the liver, bile acids are taken up through Na⁺/taurocholate cotransporting polypeptide (NTCP) and re-secreted into bile through BSEP, completing the enterohepatic circulation. Through each cycle, 95% bile acids are reabsorbed in the intestine while 5% bile acids are eliminated through fecal excretion.

In addition to the FXR signaling pathway, we have shown that BSEP is transcriptionally regulated by another nuclear receptor **liver receptor homolog 1 (LRH-1)**. LRH-1 activated the BSEP promoter during transcription and functioned as a modulator in the bile acid/FXR-mediated BSEP regulation. Our findings suggest that LRH-1 plays a supporting role with FXR in maintaining hepatic bile acid levels by coordinately regulating CYP7A1 and BSEP for bile acid synthesis and elimination, respectively.⁴

Oxysterols, oxidized derivatives of cholesterol, serve as endogenous ligands for nuclear receptor liver x receptor (LXR) to regulate cholesterol homeostasis. We discovered that oxysterols can also act as FXR ligands and induce BSEP expression.⁵ This finding demonstrates that oxysterols function as dual ligands for both LXR and FXR, and are potentially involved in regulating the biosynthesis, transport and disposition of cholesterol as well as bile acids.

Modulation of BSEP expression by xenobiotics has therapeutic or toxicological effects.

As the rate-limiting step in bile acid disposition, modulation of BSEP expression by xenobiotics, such as drugs and natural products, significantly impacts cholesterol metabolism and intrahepatic bile acid levels. Guggulsterone is a natural product from the *Commiphora mukul* tree and has been used by humans for over several thousands of years. However, the underlying mechanism is unknown. We found that guggulsterone synergistically up-regulates BSEP expression with bile acids and subsequently promotes conversion of cholesterol into bile acids.⁶ Such up-regulation of BSEP expression by guggulsterone represents a possible mechanism for the guggulsterone-mediated hypolipidemic effect.

BSEP expression is severely diminished in patients with hepatocellular carcinoma (HCC) associated with altered FXR isoform expression.

A genetic defect of BSEP leads to severe cholestasis and HCC in young children. Clinical studies showed that bile acid homeostasis is disrupted in HCC

patients with elevated serum bile acid level as a proposed marker for HCC.⁷ However, the underlying mechanisms remain largely unknown. In our study, we found that BSEP expression was severely diminished in HCC patients and was associated with altered FXR isoform (FXR α 1 and FXR α 2) expression. Further studies showed that proinflammatory cytokines **interleukin-6 (IL-6)** and **tumor necrosis factor-alpha (TNF- α)** were significantly elevated in HCC tissues. Treatment of hepatoma Huh 7 cells with IL-6 and TNF- α resulted in a marked alteration in FXR isoform expression concurrent with a significant decrease in BSEP expression. Thus restoration of BSEP expression through suppressing inflammation in the liver may re-establish the bile acid homeostasis with beneficial effects in HCC patients (submitted for publication).

Cross-talking between bile acid and estrogen signaling pathway in the induction of intrahepatic cholestasis of pregnancy (ICP) and gallstone disease (GD).

Among diseases resulting from the imbalance of bile acid levels, ICP and GD are both associated with estrogen.⁸ Although multiple risk factors are linked to those disorders, studies have demonstrated that estrogen may play a key role in the induction of the two diseases. Our research program is directly aimed at investigating the underlying mechanism for the two distinct but related diseases. We showed that estrogen repressed human BSEP expression *in vitro* and *in vivo*, and the repression was mediated by **estrogen receptor α (ER α)** through physically interacting with FXR, indicating a crosstalk between estrogen/ER α and bile acids/FXR signaling pathway (unpublished results). We plan to test the hypothesis that down-regulation of BSEP expression by estrogen is mediated through a novel non-classical transrepression pathway, a direct interaction between ER α and FXR, and as a consequence is a common risk factor for ICP and GD.

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Ruitang Deng, PhD, is an Associate Professor in the Department of Biomedical and Pharmaceutical Sciences at the University of Rhode Island College of Pharmacy.

Disclosure of Financial Interests

The author and/or their spouse/significant other have no financial interests to disclose.

CORRESPONDENCE

Ruitang Deng, PhD
Department of Biomedical and Pharmaceutical Sciences
College of Pharmacy
University of Rhode Island
7 Greenhouse Road
Kingston, RI 02881
phone: (401) 874-4950
e-mail: DengR@mail.uri.edu