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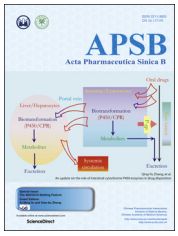

REVIEW

Fatty liver diseases, bile acids, and FXR

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Abstract The prevalence of nonalcoholic fatty liver disease (NAFLD) worldwide has increased at an alarming rate, which will likely result in enormous medical and economic burden. NAFLD presents as a spectrum of liver diseases ranging from simple steatosis, nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and even to hepatocellular carcinoma (HCC). A comprehensive understanding of the mechanism(s) of NAFLD-to-NASH transition remains elusive with various genetic and environmental susceptibility factors possibly involved. An understanding of the mechanism may provide novel strategies in the prevention and treatment to NASH. Abnormal regulation of bile acid homeostasis emerges as an important mechanism to liver injury. The bile acid homeostasis is critically regulated by the farnesoid X receptor (FXR) that is activated by bile acids. FXR has been known to exert tissue-specific effects in regulating bile acid synthesis and transport. Current investigations demonstrate FXR also plays a principle role in regulating lipid metabolism and suppressing inflammation in the liver. Therefore, the future determination of the molecular mechanism by which FXR protects the liver from developing NAFLD may shed light to the prevention and treatment of NAFLD.

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1. Introduction to nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and liver lipid metabolism

The prevalence of NAFLD worldwide has increased at an alarming rate with 30%–40% estimated in developed countries, and this global liver disease will result in enormous medical and economic burden[1]. NAFLD presents as a spectrum of liver diseases initiated with excess accumulation of lipids in the hepatocytes in the absence of excess alcohol consumption. Although many NALFD cases are benign in prognosis, it is estimated that some cases will progress from simple steatosis to NASH, fibrosis, cirrhosis and even hepatocellular carcinoma (HCC). NASH comprises dysregulation of lipid metabolism and increased inflammation, but a comprehensive understanding of the mechanism(s) of NAFLD-to-NASH transition remains elusive. Therefore, identification of the various genetic and environmental factors that contribute to the increased susceptibility to NASH may provide novel treatments to limit inflammation and fibrosis in NAFLD patients.

A considerable amount of efforts has been devoted to understand the mechanisms underlying simple steatosis-to-NASH transition. The most widely accepted theories are the "two-hit" and "multiple-hit" theories[2,3]. A common proposal in both theories is the acknowledgments of the secondary factors related to lipid/cholesterol metabolism and inflammation that precipitate NASH in NAFLD patients are critical in the transition from simple steatosis to NASH.

2. Bile acids

Bile acids are synthesized from cholesterol in the liver and the enzymatic pathways have been well defined over the last 60 years[4]. There are two major pathways to synthesize bile acids—one is the classical or neutral pathway, initiated with the enzymatic reaction of cholesterol-7α-hydroxylase (CYP7A1), and the other one is the alternative or acidic pathway initiated by CYP27A1. The classical pathway produces cholic acid (CA) and the alternative pathway generates chenodeoxycholic acid (CDCA). In rodents and other species, CDCA is rapidly converted to more hydrophilic forms of bile acids in the liver, such as muricholic acid (MCA) or ursodeoxycholic acid (UDCA). In addition to CYP7A1 and CYP27A1, there are at least two other pathways that lead to bile acid production involving cholesterol 24 and 25 hydroxylation. CA-, CDCA-, and UDCA-derived bile acids are termed primary bile acids that are conjugated in the liver to taurine or glycine. The conjugated bile acids are excreted into the bile, and, in the intestine, the intestinal microflora de-conjugate the primary bile acids and remove a hydroxylated group, generating secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA). Bile acids have been important during evolution and maybe undergone a variety of modifications[5]. Bile acids have been shown to not only serve as detergent in facilitating the intestinal absorption of lipids and lipid-soluble vitamins (vitamins A, D, K, and E), they are also critical in mediating cellular and molecular signals *via* activating nuclear receptors, FXR, pregnane X receptor, and vitamin D receptor, as well as G-protein coupled bile acid receptors, TGR5, and sphingosin-1-phosphate receptor 2[6]. The revealed roles of bile acids in regulating liver functions, intestinal health, and systemic homeostasis are diverse.

3. FXR and tissue-specific roles of FXR in regulating bile acid homeostasis

The *FXR* gene (*NR1H4/Nr1h4*) was cloned in 1995[7] and in 1999, bile acids were identified as endogenous ligands of FXR with CDCA being the strongest endogenous ligand[8–10]. The generation of *Fxr* knockout (KO) mice has accelerated the research of the roles of FXR in physiology and pathology[11]. FXR deficiency in mice renders the animals to cholestasis[11], disrupted cholesterol homeostasis[12], susceptibility to atherosclerosis in a gender-dependent manner[13], partial failure in liver regeneration[14,15], spontaneous HCC[16,17], increased NASH development[18], and an elevated susceptibility to colon cancer formation[19]. Down-regulation of FXR expression and function has also been reported in human HCC and colon cancer[20,21]. The underlying mechanisms by which FXR regulates these pathological processes appear to be due to either FXR-mediated, direct transcriptional regulation of critical mediators in the disease development, or *via* improving bile acid homeostasis, as increased bile acid levels have been shown to promote inflammation and tumorigenesis.

FXR exerts bile-acid regulatory effects *via* a tissue-specific mechanism. The activation of FXR in the liver and more critically, in the intestine, is critical in the feedback suppression of bile acid synthesis[22–25]. In the intestine, activation of FXR induces the expression of an endocrine fibroblast growth factor 15 (FGF15, human homolog, FGF19), and the increased expression of FGF15 has been shown to mediate direct effects on suppressing bile acid synthesis and promote hepatic health[23,24,26].

4. Tissue specific roles of FXR in regulating lipid metabolism and inflammation

The tissue specific effects of FXR have provided an interesting perspective to study liver-intestine axis[24,27]. Clear tissue-specific roles of FXR have been proposed by genome-wide DNA ChIP-seq technology in mice[28]. It is very interesting to observe that there is only 11% overlap between FXR-binding sites between the liver and intestine, indicating underlying molecular mechanism of tissue-specific regulation by FXR activation. Furthermore, the annotated pathways in the liver and intestine predict differential functions of FXR in the liver and intestine. The detailed clarification of FXR functions in liver and intestine awaits further research. The human FXR genome-wide binding has been reported which supports the view that mice can be used as a model to study human FXR functions, particularly in diseases involved in lipid dysregulation and inflammation[29]. This is because the comparison between FXR genome-wide binding in mouse livers and in human primary hepatocytes reveals that FXR regulates a few conservative pathways in both species. FXR activation has been known to reduce triglyceride levels *via* suppressing *de novo* synthesis and uptake of fatty acids in the liver[30], and recently, the roles of FXR in reducing inflammation have been emerging[31]. The FXR expression and function were reduced during acute phase response[32]. FXR activation strongly induced the expression of genes involved in anti-inflammation, such as kininogen[33], facilitated the suppression of C-reactive protein production by interleukin 6 (IL-6)[34], inhibited smooth muscle inflammation[35], suppressed nuclear factor κB (NF-κB) activation in the liver[36], reduced liver injury during systemic lupus erythematosus[37], decreased inflammation induced

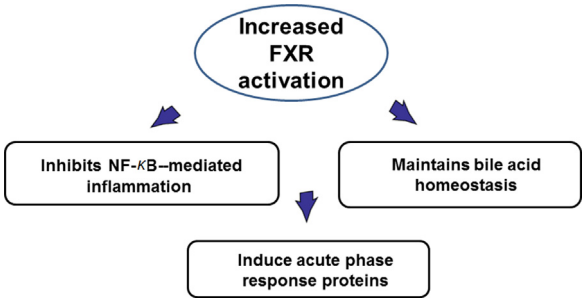

Figure 1 Proposed roles of hepatic FXR in anti-inflammation in the liver. FXR may exert its anti-inflammatory effects *via* (1) antagonizing NF-κB function; (2) maintaining bile acid homeostasis; and (3) inducing acute phase response proteins.

by myofibroblasts[38], induced acute phase proteins[39], and decreased inflammation in the intestine[40]. In the liver, the molecular mechanism by which FXR antagonizes inflammation may be due to a acetyl/small ubiquitin-like modifier (SUMO) switch so that SUMOylation of FXR increased FXR's ability to suppress NF-κB–mediated transcriptional induction of inflammatory genes[41]. But the role of FXR in inflammation has also been reported to be controversial[42], perhaps reflecting the complexity of FXR in modulating inflammation and immune function under different diseases and/or different disease stages. The roles of FXR in the fight against liver inflammation have been summarized in Fig. 1.

5. Future perspectives

Recently, a role of tissue-specific FXR functions in regulating fatty liver disease is emerging. It is reported that intestinal antagonism of FXR function inhibits sterol regulatory element-binding protein-1c (SREBP-1c) function by reducing the production of ceramide in the intestine[43,44], indicating that intestinal inhibition/antagonism of FXR may present a promising opportunity in the treatment of liver steatosis. However, intestine-specific activation of FXR led to enhanced adipose tissue browning, which increased insulin sensitivity and reduced obesity[45]. These conflicting, interesting reports prompt further detailed studies of FXR's tissue specific effects on fatty liver formation, NASH development and metabolic syndrome progression. The recent development of FXR synthetic modulators in the treatment of NASH and cholestasis also urge us to careful define the functions of FXR in a tissue (cell)-specific, as well as species-dependent, manner.

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