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Minireview

FXR signaling in metabolic disease

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Abstract **Farnesoid X receptor (FXR), a member of the nuclear receptor superfamily, has been shown to be important in controlling numerous metabolic pathways; these include roles in maintaining bile acid, lipid and glucose homeostasis, in preventing intestinal bacterial infection and gallstone formation and in modulating liver regeneration and tumorigenesis. The accumulating data suggest that FXR may be a pharmaceutical target for the treatment of certain metabolic diseases.**

Keywords: FXR; Bile acid; Lipid; Glucose

1. Introduction

Farnesoid X receptor (FXRα, NR1H4), a member of the nuclear receptor (NR) superfamily, was originally cloned in 1995 [1,2]. The conservation of FXRα amino acid sequence from teleost fish to humans suggests a critical role for this nuclear receptor in numerous species. Nuclear receptors are transcriptional factors that are involved in several diverse physiological functions that include reproduction, development and metabolism [3]. The human genome contains 48 nuclear receptors. Most of these nuclear receptors have a poorly-defined N-terminal activation function domain 1 (AF1), a highly conserved DNA binding domain (DBD), a hinge region that links the DBD to a ligand-binding domain (LBD) that also contains a strong transcriptional activation function domain 2 (AF2). In general, nuclear receptors bind as monomers or dimers to specific DNA sequences termed hormone response elements and regulate gene expression. Transcriptional activation is often stimulated when agonists (usually small lipophilic molecules) bind to the pocket formed by the LBD, and alter the conformation of the nuclear receptor; the result is often release of co-repressors, recruitment of co-activators and activated gene transcription [3,4]. Specific agonists include steroid hormones, thyroid hormone, 1,25-dihydroxyvitamin D3, fatty acids, oxysterols, retinoic acids, phospholipids and bile acids. However, certain members of the nuclear receptor family function independent of a ligand and/or regulate transcription by processes that do not involve binding to DNA. The latter receptors may either lack a DBD, or a ligand has yet to be identified or they bind to and inhibit other transcription factors without themselves interacting with DNA. Small heterodimer partner (SHP; NR0B2) is but one example of a nuclear receptor that lacks a DBD and represses gene transcription by binding to and inhibiting other transcription factors [5].

FXRα expression is limited to very few tissues; it is highly expressed in the liver, intestine, kidney and adrenal gland [1,2,6,7]. In contrast, low FXR mRNA levels were detected in white adipose tissue and heart [6,8,9]. Whether FXR is functional and controls gene expression in vivo in the latter two tissues remains to be determined. The single human or mouse gene encodes four isoforms (FXRα1, FXRα2, FXRα3 and FXRα4), as a result of the use of two different promoters and alternative splicing between exons 5 and 6 [6,7]. Compared to FXRα1 and FXRα2, FXRα3 and FXRα4 mRNAs are smaller but encode proteins that contain an extended N-terminus. In addition, FXRα1 and FXRα3, but not FXRα2 and FXRα4, contain a four amino acid (MYTG) insert immediately adjacent to the DBD. Although many genes are regulated equally well in vitro by all four FXR isoforms, certain genes, that include ileum bile acid-binding protein (I-BABP), syndecan-1, αA-crystallin and fibroblast growth factor 19 (FGF19) are more responsive to FXRα2 and FXRα4 (the isoforms that lack the MYTG insert) (reviewed in [10]). Despite the identification of four FXR isoforms, the physiological importance of gene regulation by each isoform remains to be established. Rodents, rabbits and dogs contain a second *Fxr* gene, *Fxr*β (NR1H5), that is a pseudogene in humans and primates. In this review, we use the term FXR to refer specifically to FXRα.

FXR, like a subset of nuclear receptors, binds to specific response elements as a heterodimer with retinoic X receptor (RXR, NR2B1). The FXR response element (FXRE) contains two copies of a consensus sequence (AGGTCA) arranged as inverted repeats separated by one nucleotide (IR1) or everted repeats separated by 8 nucleotides (ER8) or direct repeats separated by four nucleotides (DR4) [11,12]. FXR has also been reported to bind DNA as a monomer [13], although this appears to be a rare event.

A number of excellent reviews on FXR and/or bile acid metabolism have been published recently [10–12,14,15]. Consequently, we propose here to emphasize the more recent advances that have delineated a role for FXR in bile acid, lipoprotein and glucose metabolism, liver regeneration, bacterial growth and tumor growth. Based on the many metabolic

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pathways that are regulated by FXR, it seems possible that specific agonists may prove useful in the treatment of cholestasis, hyperlipidemia, diabetes and/or cholesterol gallstone disease.

2. FXR agonists

In 1999 bile acids were identified as the endogenous ligands that bind to and activate FXR [16–18]. The order of potency of bile acids is chenodeoxycholic acid (CDCA) > lithocholic acid (LCA) = deoxycholic acid (DCA) > cholic acid (CA). More recently, androsterone was reported to function as an endogenous (although very weak) ligand for FXR [19].

However, it is now clear that bile acids are promiscuous activators of nuclear receptors since they not only activate FXR, but also activate the pregnane X receptor (PXR), vitamin D receptor (VDR) and the constitutive androstane receptor (CAR) [10]. In addition, bile acids regulate c-Jun N-terminal kinase (JNK) cascade and the mitogen-activated protein kinase pathway, independent of nuclear receptor activation. Finally, bile acids have recently been shown to activate TGR5, a G-protein coupled receptor [20]. Thus, bile acids can modulate numerous metabolic pathways by various mechanisms (reviewed in [10]).

The development of potent, specific FXR agonists, such as GW4064 [21], fexaramine [22], AGN34 [23] and 6α-ethyl-chenodeoxycholic acid (6-ECDCA) [24], and the generation of FXR-deficient mice [25] have provided powerful tools to investigate the role of FXR in controlling diverse metabolic pathways. Because bile acids are promiscuous activators of many pathways, such tools were particularly important in delineating FXR-dependent and -independent pathways.

3. Regulation of FXR expression and activity

Little is known about the regulation of the FXR gene *per se*. Hepatic FXR mRNA levels have been shown to be induced by prolonged fasting [26]. Interestingly, peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), which plays an important role in glucose and energy homeostasis [27], is also induced during the fast. FXR mRNA levels were also increased following overexpression of PGC-1α in primary hepatocytes [26]. This latter induction of FXR likely results from PGC-1α coactivation of HNF4α bound to DR-1 elements in the two FXR promoters [26]. In addition, PGC-1α may also serve as a coactivator for FXR [26,28,29]. In other studies, protein arginine methyl-transferase type I (PRMT1) [30], TRRAP [31], and DRIP205 [32] have been identified as FXR-interacting proteins that likely function as coactivators of the nuclear receptor. Together, these data suggest that hepatic FXR may be regulated by the nutritional status and might be particularly responsive to the coactivator PGC-1α.

Recent studies have shown FXR mRNA levels are increased in cultured cells in response to high levels of glucose [33]. This stimulation may be of physiological importance since hepatic FXR levels are elevated in diabetic db/db mice [34]. However, Duran-Sandoval et al. have reported that hepatic FXR mRNA levels are repressed in both streptozotocin (STZ)-induced diabetic rats and aging diabetic Zucker rats [33]. The reasons for these apparent discrepancies remain unknown at the present time.

4. FXR and the regulation of bile acid metabolism

Bile acids, the end products of hepatic cholesterol catabolism, are important for lipid digestion and absorption from the intestinal lumen, serve as signaling molecules, and also represent the principal means of eliminating cholesterol from the body. Importantly, in order to maintain whole body cholesterol homeostasis, approximately 5% of the bile acids secreted from the gall bladder into the duodenum fail to be reabsorbed and are excreted in the feces.

4.1. FXR and bile acid synthesis

Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the classic pathway of bile acid synthesis [14]. It has been known for many years that bile acids returning to the liver via the enterohepatic circulation are capable of repressing their own synthesis. The molecular mechanisms involved in this repression appear to involve at least three pathways; SHP, mouse fibroblast growth factor (FGF)15/human FGF19 and JNK. Activation of hepatic FXR, in response to bile acids returning to the liver after re-absorption from the ileum, increases the expression of the FXR target gene SHP. SHP in turn binds to and inactivates liver receptor homolog 1 (LRH-1, NR5A2), a transcription factor that is important for *Cyp7a1* expression. The result is SHP-dependent transcriptional repression of *Cyp7a1* [35,36]. Unexpectedly, hepatic *Cyp7a1* mRNA levels were repressed following treatment of $Shp^{-/-}$ mice with bile acids [37,38]. These latter data indicate that other pathway(s), independent of SHP, are also involved in the repression of *Cyp7a1* by bile acids.

Recent studies have identified a second pathway that involves FGF15; activation of intestinal FXR by GW4064 or bile acids increases the expression and secretion of FGF15 from enterocytes. Secreted FGF15 subsequently binds to the receptor FGFR4, localized on the plasma membrane of hepatocytes, resulting in activation of the JNK pathway and repression of *Cyp7a1* [39]. The finding that $Fgfr4^{-/-}$ mice have increased bile acid pools and increased *Cyp7a1* expression is entirely consistent with the critical role of *Fgfr4* [40]. Bile acids may also repress *Cyp7a1* via direct activation of the JNK pathway [41]. Together, these three pathways likely account for the regulation of *Cyp7a1* and the control of bile acid synthesis from cholesterol.

4.2. FXR and bile acid conjugation, secretion and absorption

Once synthesized, bile acids are conjugated to taurine or glycine prior to secretion across the canalicular membrane of the hepatocytes. These reactions are catalyzed by bile acid-CoA synthetase (BACS) and bile acid-CoA:amino acid *N*-acetyltransferase (BAT). FXR directly regulates both genes. In addition, FXR also regulates uridine 5′-diphosphate glucuronosyltransferase 2B4 (UGT2B4) and dehydroepiandrosterone-sulfotransferase (STD; SULT2A1) (reviewed in [10]). UGT2B4 converts hydrophobic bile acids into more hydrophilic glucuronide derivatives, whereas SULT2A1 is a hydroxysteroid sulfo-conjugating enzyme. The up-regulation of BACS, BAT, UGT2B4 and SULT2A1 suggests that FXR activation

promotes numerous enzymes involved in bile acid conjugation that precedes secretion.

Activated FXR also increases the transcription of three hepatic transporters that function to efflux bile acids out of hepatocytes (reviewed in [10,12]); the three proteins, bile salt export protein (BSEP), the multidrug resistant-associated protein 2 (MRP2, ABCC2) and the multidrug resistance P-glycoprotein 3 (MDR3, ABCB4) are localized on the bile canalicular membrane of hepatocytes and secrete bile acids (and other compounds) from the hepatocytes into the bile canaliculi. Bile, containing bile acids, phospholipids, cholesterol and some proteins, is then stored in the gallbladder.

Entry of food into the intestine causes the release of cholecystokinin (CCK) from the proximal duodenum. CCK stimulates the gall bladder to contract and expel bile into the duodenum where it facilitates lipid digestion and absorption. About 95% of the bile acids are re-absorbed in the distal ileum via the apical sodium-dependent bile acid transporter (ASBT). Ileum bile acid-binding protein (I-BABP) binds bile acids and may protect the enterocytes from the effects of high levels of these detergents prior to their being pumped across the basolateral membrane to the portal circulation via heterodimeric organic solute transporter-α and -β (OSTα, OSTβ). FXR has been shown to directly regulate ASBT, IBABP and both OST genes [10].

In a particularly insightful study, Kliewer and colleagues recently demonstrated that the normal filling of the gall bladders was dependent upon the FXR target gene FGF15 [42]. They demonstrated that the refilling of the gall bladder after CCK-stimulated emptying is dependent upon the synthesis and secretion of FGF15 from the distal ileum. Importantly, these authors provided data to support a model in which bile acids that are reabsorbed in the distal ileum, induce FGF15 expression by activating FXR in the enterocytes. The secreted FGF15 subsequently binds to FGF15 receptors on the gall bladder and promotes relaxation of gall bladder smooth muscle to allow refilling [42]. Since poor gall bladder motility has been linked to gall stone formation, the authors suggest that changes in FGF15 expression may prove clinically useful for treatment of this disease that afflicts millions of people in the USA [42].

Thus, it is clear that FXR is a key sensor for bile acids and has a central role in maintaining bile acid homeostasis, as it regulates all aspects of bile acid metabolism, including bile acid synthesis, conjugation, secretion, absorption and refilling of the gall bladder.

4.3. FXR and cholesterol gallstone disease

FXR has recently been shown to play a role in gallstone formation. By using quantitative trait locus analysis, Wittenburg et al. proposed that FXR and ABCG5/ABCG8 are possible determinants of cholesterol gallstone disease [43]. Subsequently, Moschetta et al. demonstrated that, compared to wild-type mice, $Fxr^{-/-}$ mice were more susceptible to cholesterol gallstone formation following administration of a lithogenic diet [44]. Importantly, gallstone formation was reduced when gallstone-susceptible C57L mice were treated with the FXR agonist GW4064 [44]. The authors proposed that this protection likely resulted from induction of *Bsep* and *Mdr2* and increased transport of bile acids from the liver into bile. Such increased levels of bile acids would be expected to prevent or reduce cholesterol crystallization from the bile [44]. These studies open up the possibility that FXR agonists may prove useful in the treatment of cholesterol gallstone disease.

5. FXR and lipid metabolism

It has been known for many years that the bile acid pool size has a profound effect on lipid metabolism [45–47]. The reduced bile acid pool, following either administration of bile acid-binding resins (e.g. cholestyramine or cholestipol) or ileal surgery, results in reduced levels of plasma LDL and increased plasma triglyceride and HDL. The finding that administration of bile acids (CDCA or CA) to humans or animals results in reduced plasma triglyceride and HDL levels and increased LDL is entirely consistent with a key role for bile acids in controlling plasma lipids (reviewed in [48]). Studies with $Fxr^{-/-}$ mice or following administration of FXR-specific agonists have demonstrated that FXR plays a central role in controlling lipid homeostasis.

5.1. FXR and triglyceride metabolism

Recent data have demonstrated that activation of FXR reduces both hepatic lipogenesis and plasma triglyceride and cholesterol levels. As detailed below, FXR activation induces genes involved in lipoprotein metabolism/clearance and represses hepatic genes involved in the synthesis of triglycerides (Fig. 1). For example, treatment of mice with FXR agonists results in the repression of *Srebp-1c* mRNA levels in murine livers or isolated murine primary hepatocytes [26,49]. This repression was not observed with $Shp^{-/-}$ mice, suggesting that SHP, a known FXR target gene, is required for the repression [49]. Since SREBP-1c functions as a critical transcription factor that regulates many genes involved in both fatty acid and triglyceride synthesis, it is not surprising that hepatic triglyceride synthesis and secretion is reduced following the repression of *Srebp-1c* by FXR. However, the finding that $Fxr^{-/-}$ mice have normal or even reduced hepatic SREBP-1c expression [26,50] suggests that FXR-independent mechanisms must also be important.

Activation of FXR also results in increased hepatic expression of receptors (VLDL receptor and syndecan-1) that are involved in lipoprotein clearance and increased apoC-II that co-activates lipoprotein lipase (LPL). In addition FXR activation results in decreased expression of proteins (apoC-III and ANGTPL3) (reviewed in [10]) that normally function as inhibitors of LPL. Finally, FXR induces human PPARα [51], a nuclear receptor that functions to promote fatty acid β-oxidation. Taken together, these data suggest that FXR activation lowers plasma triglyceride levels via both repressing hepatic lipogenesis and triglyceride secretion, and increasing the clearance of triglyceride-rich lipoproteins from the blood (Fig. 1).

5.2. FXR and cholesterol metabolism

Treatment of mice with a specific FXR agonist results in a decline in both plasma triglyceride and cholesterol. The observation that $Fxr^{-/-}$ mice have increased plasma LDL and HDL levels is consistent with an important role for FXR in controlling plasma lipoprotein levels [25,52]. The finding that $Fxr^{-/-}$ mice have reduced hepatic expression of scavenger receptor, type I B1 (SR-BI) [52], a receptor that is thought to be impor-

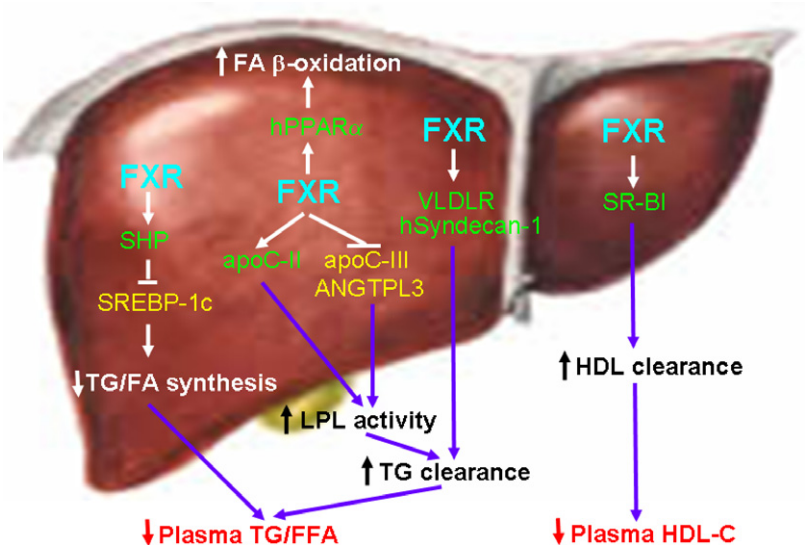

Fig. 1. Regulation of lipid homeostasis by hepatic FXR. Activation of hepatic FXR lowers plasma free fatty acid (FFA) and triglyceride (TG), likely resulting from (i) repression of hepatic TG and fatty acid (FA) synthesis as a result of SHP-dependent inhibition of SREBP-1c; (ii) induction of apoC-II and repression of apoC-III and ANGTPL3 in the liver, resulting in enhanced lipoprotein lipase (LPL) activity; (iii) induction of VLDL receptor (VLDLR) and human syndecan-1 (hSyndecan-1), thus promoting clearance of TG-rich lipoproteins; and (iv) induction of human PPARα and FA β-oxidation. In addition, FXR activation lowers plasma HDL cholesterol levels likely through the induction of hepatic SR-BI and increased clearance of HDL. FXR-induced or -repressed genes are shown in green or yellow, respectively. The mechanisms of repression remain largely unknown.

tant in the delivery of HDL cholesterol to the liver as part of the reverse cholesterol transport pathway, suggests that this pathway may be impaired in $Fxr^{-/-}$ mice. In agreement with this observation, FXR activation was reported to induce hepatic SR-BI expression [34]. Thus SR-BI may play a role in FXR-controlled cholesterol homeostasis (Fig. 1).

5.3. FXR and atherosclerosis

The original observation that $Fxr^{-/-}$ mice have a pro-atherogenic lipoprotein profile (increased plasma triglyceride, free fatty acids and LDL) [25] suggested that these mice may have altered susceptibility to atherogenesis. However, $Fxr^{-/-}$ mice on a chow or western diet fail to develop atherosclerotic lesions (our unpublished data). To better assess the possible role of FXR in atherogenesis, double knockout mice, either $Fxr^{-/-}apoE^{-/-}$ [53,54] or $Fxr^{-/-}Ldlr^{-/-}$ [55], were generated and fed a western diet. Surprisingly, compared to $Ldlr^{-/-}$ mice, male $Fxr^{-/-}Ldlr^{-/-}$ mice had decreased aortic atherosclerotic lesions [55]. The decrease may be a result of decreased LDL levels in the double knockout mice [55]. The mechanism resulting in this reduction in LDL remains unknown. Guo et al. also observed decreased lesions in $Fxr^{-/-}apoE^{-/-}$ mice compared to the $apoE^{-/-}$ control mice [54]. Thus, loss of FXR unexpectedly resulted in reduced levels of atherosclerosis in two different double knockout mouse models of atherosclerosis. However, in a third independent investigation, Hanniman et al. reported that administration of a western diet to $Fxr^{-/-}apoE^{-/-}$ mice resulted in increased atherosclerotic lesions as compared to $apoE^{-/-}$ control mice [53]. At present, the different results reported in the latter two studies with $Fxr^{-/-}apoE^{-/-}$ mice remain unexplained.

Using a different approach, Bishop-Bailey et al. used immunohistochemistry and concluded that FXR is expressed in atherosclerotic lesions and vascular smooth muscle cells of human vessels [56]. In contrast, we have failed to identify FXR transcripts in macrophages or extracts from murine aortas (our unpublished data). In addition, the level of FXR in human umbilical vein endothelial cells (HUVEC) and human aortic endothelial cells (HOVEC) was reported to be approximately 1/1000 of that in HepG2 cells [57]. Consequently it remains to be established whether such low levels of FXR are sufficient to regulate expression of known FXR target genes. The finding that CDCA treatment caused reduced expression of endothelin-1 [58], a vasoconstrictive peptide, and increased expression of the adhesion molecules ICAM-1 and VCAM-1 [57] is of interest. However, it remains possible that such regulation may involve bile acid-regulated but FXR-independent pathways.

6. FXR and glucose metabolism

Recent studies demonstrated that FXR also plays a significant role in regulating glucose homeostasis. Treatment of mice with the FXR agonist GW4064 or cholic acid, or following infection with adenovirus that expresses a constitutively active FXR-VP16 fusion protein, resulted in a significant reduction of plasma glucose levels and improved insulin sensitivity [8,34,59]. These effects were noted in three different diabetic models (db/db, ob/ob or KK-A(y) mice). The finding that over-expression of constitutively active FXR in the liver, as a result of adenoviral infection, is consistent with a key role for hepatic, rather than extra-hepatic FXR. As detailed below, activation of hepatic FXR regulates gluconeogenesis, glycogen synthesis and insulin sensitivity (Fig. 2).

6.1. FXR and hepatic gluconeogenesis

Hepatic gluconeogenesis plays an important role in maintaining glucose homeostasis. The finding that plasma glucose levels of db/db mice declined approximately 50% after hepatic

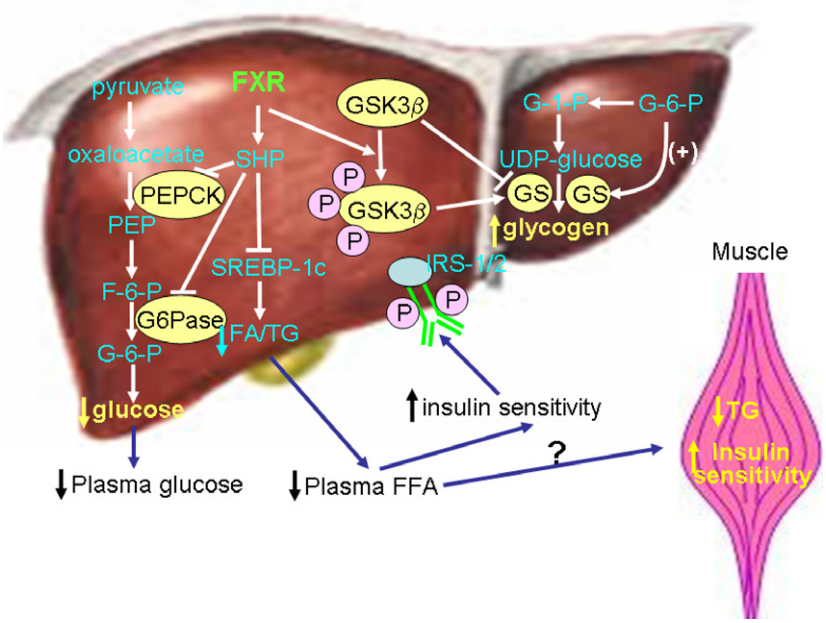

Fig. 2. Regulation of glucose homeostasis by activated FXR. Activation of hepatic FXR results in repression of PEPCK and G6Pase and increased phosphorylation of GSK3β. Since phosphorylated GSK3β is inactive, the result is increased levels of the dephosphorylated/active glycogen synthase (GS). Glucose 6 phosphate (G-6-P) also activates GS. Overall, these changes result in decreased hepatic gluconeogenesis, decreased plasma glucose levels and increased hepatic glycogen synthesis. FXR activation also results in increased phosphorylation of hepatic IRS-1 and IRS-2 and increased insulin sensitivity/signaling. It is currently not known whether the increased insulin sensitivity in the liver and muscle is a result of the reduction in the plasma levels of free fatty acids (FFA) that occur as a result of inhibition of SREBP-1c (see also Fig. 1). $Fxr^{-/-}$ mice display insulin resistance and changes in muscle lipid levels and insulin signaling. Whether these changes in $Fxr^{-/-}$ mice are a result of increased plasma FFAs is unknown at this time. PEP, phosphoenoylpyruvate. F-6-P, fructose-6-phosphate.

expression of constitutively activated FXR [34] suggested a key role for hepatic FXR. A similar decline in plasma glucose and free fatty acids was noted following administration of GW4064 to db/db or KK-A(y) mice [34]. Plasma glucose levels also declined following treatment of wild-type mice with GW4064 [34] or bile acids [59]. The latter treatments are known to activate intestinal, hepatic and renal FXR and thus do not identify the tissue(s) responsible for the hypoglycemic effect. The finding that activation of FXR repressed hepatic phosphoenoylpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) mRNA levels [34,59] is consistent with the hypoglycemic effects. It is possible that the repression of PEPCK and G6Pase following FXR activation in diabetic mice results, at least in part, from increased hepatic insulin sensitivity and reduced plasma free fatty acid levels [34]. There is also evidence to suggest that cholic acid administered in the diet reduces hepatic gluconeogenic genes through an FXR/SHP pathway [59].

In contrast to studies with diabetic mice, treatment of wild-type mice with FXR agonists has resulted in inconsistent results (stimulation [34,60] or repression of PEPCK [59,61,62], decreased or unchanged glucose levels [34,59,60]). The reason for these discrepancies remains to be determined, but might depend on efficacy of treatment or genetic backgrounds of the mice.

6.2. FXR and hepatic glycogen synthesis

The decreased hepatic expression of G6Pase following FXR activation suggested that hepatic glycogen synthesis might be altered. Consistent with this proposal, activation of FXR in either murine primary hepatocytes or the livers of diabetic db/db mice resulted in increased conversion of D-glucose to glycogen or increased hepatic glycogen levels [34]. These changes were associated with increased phosphorylation of GSK3β [34], a known key regulator of glycogen synthase. Not surprisingly, $Fxr^{-/-}$ mice have reduced hepatic glycogen content [63]. Currently, it is not known whether long term FXR activation (>two weeks) would be deleterious as a result of increased glycogen storage.

6.3. FXR and insulin sensitivity

$Fxr^{-/-}$ mice show impaired glucose tolerance and insulin sensitivity compared to wild-type mice [8,34,59]. Hyperinsulinemic-euglycemic clamp studies reveal that $Fxr^{-/-}$ mice also display a peripheral insulin resistance [8,59]. Consistent with this observation, insulin signaling was found to be impaired in the liver [59], muscle [8,59] and white adipose tissue of $Fxr^{-/-}$ mice [8]. Such changes may result from the elevated free fatty acids noted in the plasma of $Fxr^{-/-}$ mice. Importantly, glucose tolerance and insulin sensitivity were significantly improved when db/db [34] or ob/ob [8] mice were either treated with GW4064 or infected with adenovirus expressing FXR-VP16. The finding that activation of FXR significantly lowered plasma glucose, triglyceride, cholesterol and free fatty acid levels in diabetic mouse models [34], suggests that FXR agonists might prove useful in the treatment of hyperglycemia and hyperlipidemia that are observed in patients with type 2 diabetes.

Recently, FXR activation was reported to improve insulin signaling and insulin-stimulated glucose uptake in differentiated 3T3-L1 cells [8]. However, FXR is expressed at a very

low level in white adipose tissue and no FXR target gene has been identified in this tissue to date. Thus the physiological importance of FXR in the direct regulation of insulin sensitivity in white adipose tissue awaits additional studies.

7. FXR and intestinal bacterial growth

Obstruction of bile flow in humans or rodents causes intestinal bacterial growth, mucosal injury and bacterial translocation. In contrast, bile acid administration can prevent intestinal bacterial growth and translocation [64,65]. Recently, Inagaki et al. reported that FXR plays a critical role in these processes [66]. They demonstrated that FXR activation induced a number of intestinal genes, including angiogenin, inducible nitric oxide synthase and IL-18, all of which are involved in enteroprotection [66]. The finding that $Fxr^{-/-}$ mice have bacterial overgrowth in their ileum and a compromised epithelial barrier [66] is entirely consistent with the proposal that FXR is critical for controlling intestinal bacterial growth and maintaining a competent barrier. These novel studies suggest that the development of FXR agonists could provide a novel mechanism for controlling intestinal bacterial growth.

8. FXR and liver regeneration

Rodent liver has a remarkable ability to regenerate following partial hepatectomy/damage. Huang et al. recently concluded that bile acids have a novel role in this process; they reported that a diet containing 0.2% cholic acid stimulated liver regeneration in partially hepatectomized mice, while diets containing the bile acid sequestrant cholestyramine impaired liver regeneration [67]. Such sequestrants bind bile acids in the intestinal lumen and increase their excretion to the feces by preventing their reabsorption from the intestinal lumen. Consistent with these data, $Fxr^{-/-}$ mice had impaired liver regeneration ability [67]. Bile acids were shown to be required for the induction of the proliferation factor FoxM1b [67]. However, since bile acids can activate both FXR-dependent and -independent pathways, it remains to be established whether a specific FXR agonist, such as GW4064, will have a similar role in liver regeneration.

9. FXR and liver tumorigenesis

Recently, FXR was reported to play a role in tumor growth. At 12 months of age, both male and female $Fxr^{-/-}$ mice showed a high incidence of liver tumors, including hepatocellular adenoma, carcinoma and hepatocholangiocellular carcinoma [68,69]. These $Fxr^{-/-}$ mice also exhibit elevated expression of genes involved in inflammation and cell cycle and elevated plasma bile acid levels [68,69]. Consistent with these observations, administration of a diet supplemented with 0.2% cholic acid diet promoted *N*-nitrosodiethylamine-initiated liver tumorigenesis, whereas feeding a diet containing the bile acid sequestrant cholestyramine to lower the bile acid pool size in the $Fxr^{-/-}$ mice, significantly reduced the incidence of liver tumorigenesis [69]. Together, these observations suggest a link between bile acid homeostasis, FXR and hepatic tumorigenesis.

10. FXR and cholestasis

To date, mutations or polymorphisms in FXR have not been directly linked to any human disease. However, a number of FXR target genes have been implicated in several inherited cholestatic liver disorders.

FIC1 (ATP8B1) is a P-type ATPase and functions as an aminophospholipid flippase. Mutations in the FIC1 gene cause both progressive familial intrahepatic cholestasis type 1 (PFIC-1) and benign recurrent intrahepatic cholestasis type 1 (BRIC-1) depending on the mutation [70]. Recent studies have reported that decreased FXR expression and activity is associated with FIC1 mutations [71,72], suggesting that FXR may play an important role in the pathogenesis of PFIC-1. PFIC-2 results from mutations in the FXR target gene BSEP [73], and is phenotypically similar to PFIC-1. PFIC-3 results from mutations in MDR3 [74,75], another FXR target gene [10], and is characterized by high serum γ-glutamyltranspeptidase activity, absence of phosphatidylcholine in the bile, bile ductular proliferation and biliary fibrosis.

FXR activation has provided beneficial effects on several experimental cholestatic rodent models. Both GW4064 and 6-ECDCA are shown to protect hepatocytes from cholestasis induced by α-naphthylisothiocyanate and estrogen [76,77]. Both GW4064 and 6-ECDCA induce *Bsep*, *Mrp2* and *Mrd2*, and reverse ductular proliferation and necrosis [76,77]. Interestingly, a recent report shows that $Fxr^{-/-}$ mice are protected from obstructive cholestasis [78]. Together, these studies suggest that changes in FXR expression and activity may affect numerous genes that are associated with cholestasis.

11. Conclusion

The utilization of both FXR agonists and $Fxr^{-/-}$ mice have demonstrated that FXR signaling modulates many metabolic pathways. The current data suggest that FXR agonists may be useful in the treatment of type 2 diabetes, hypertriglyceridemia, certain cholestasis and cholesterol gallstone disease. However, to our knowledge, no reports on the clinical use of synthetic FXR agonists have been reported to date. The development of novel full or partial FXR agonists, that are easily absorbed after oral administration, may provide the appropriate tools to determine whether FXR activation is beneficial for patients with specific metabolic disorders.

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