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Targeting nuclear receptors for the treatment of fatty liver disease

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Abstract

Ligand-activated nuclear receptors, including peroxisome proliferator-activated receptor alpha (PPAR α), pregnane X receptor, and constitutive androstane receptor, were first identified as key regulators of the responses against chemical toxicants. However, numerous studies using mouse disease models and human samples have revealed critical roles for these receptors and others, such as PPAR β/δ , PPAR γ , farnesoid X receptor (FXR), and liver X receptor (LXR), in maintaining nutrient/energy homeostasis in part through modulation of the gut-liver-adipose axis. Recently, disorders associated with disrupted nutrient/energy homeostasis, e.g., obesity, metabolic syndrome, and non-alcoholic fatty liver disease (NAFLD), are increasing worldwide. Notably, in NAFLD, a progressive subtype exists, designated as non-alcoholic steatohepatitis (NASH) that is characterized by typical histological features resembling alcoholic steatohepatitis (ASH), and NASH/ASH are recognized as major causes of hepatitis virus-unrelated liver cirrhosis and hepatocellular carcinoma. Since hepatic steatosis is basically caused by an imbalance between fat/energy influx and utilization, abnormal signaling of these nuclear receptors contribute to the pathogenesis of fatty liver disease. Standard therapeutic interventions have not been fully established for fatty liver disease, but some new agents that activate or inhibit nuclear receptor signaling have shown promise as possible therapeutic targets. In this review, we summarize recent findings on the roles of nuclear receptors in fatty liver disease and discuss future perspectives to develop promising pharmacological strategies targeting nuclear receptors for NAFLD/NASH.

Keywords

Peroxisome proliferator-activated receptor; Energy vector; Steatohepatitis; Liver fibrosis; Hepatocellular carcinoma; Tissue-specific agonist/antagonist

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

1. Introduction

1.1. Liver as a main regulator of whole-body metabolism

Liver is the largest solid organ in the body playing a crucial role in maintaining energy homeostasis through metabolism of various nutrients. For example, the main symptoms and signs of acute liver failure are jaundice (impaired bilirubin conjugation/excretion), bleeding tendency (impaired synthesis of coagulation factors), and consciousness disturbance (impaired detoxification of ammonia and other neurotoxic metabolites). Patients having liver cirrhosis often exhibit impaired glucose metabolism (insulin resistance and diabetes), protein/amino acid metabolism (decreased albumin and branched-chain amino acids and increased aromatic amino acids), and lipid metabolism (hypercholesterolemia). These clinical findings mirror a key role of the liver in whole-body metabolism.

Infection with hepatotropic viruses and parasites, autoimmunity, in-take of ethanol and certain drugs/medications, and exposure to occupational and environmental toxicants cause liver damage. In Asia, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are among the main causes for chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Recent advances in antiviral therapies, such as nucleoside analogues for HBV and pegylated interferon/ribavirin and direct-acting antiviral agents for HCV, have improved the quality of life and survival for HBV- or HCV-infected patients. Further improvements in preventive/therapeutic strategies will lead to reduced incidence and mortality of patients having hepatitis virus-related diseases.

Metabolic derangements also cause chronic liver disease, such as fatty liver disease, glycogen storage disease, and hemochromatosis. Fatty liver disease refers to a pathological spectrum ranging from lipid accumulation in hepatocytes (steatosis) to the development of accompanying hepatocyte degeneration (ballooning, Mallory-Denk body) and hepatic inflammation (steatohepatitis), eventually leading to liver fibrosis/cirrhosis, portal hypertension, decompensated liver failure, and HCC (Cohen, Horton, & Hobbs, 2011). Although the mechanism of fatty liver disease may vary according to the etiologies, the liver pathology is often indistinguishable. Therefore, fatty liver diseases are classified according to their etiology/cause, i.e., long-term excess ethanol consumption [alcoholic liver disease/steatohepatitis (ALD/ASH)], over-nutrition, visceral obesity, and metabolic syndrome without ethanol in-take [non-alcoholic fatty liver disease/steatohepatitis (NAFLD/NASH)], occupational/environmental chemical exposure [toxicant-associated fatty liver disease/steatohepatitis], and others (e.g., drug-induced steatohepatitis and steatohepatitis following gastrointestinal surgery).

1.2. NAFLD/NASH - emerging liver disease

The worldwide spread of sedentary lifestyle and diet westernization has increased a prevalence of NAFLD in many countries among wider generations. In Japan, approximately 30% of Japanese upon annual health checkups were found to have NAFLD (Kojima, Watanabe, Numata, Ogawa, & Matsuzaki, 2003; Eguchi et al., 2012), which extrapolates to an estimated 20 million NAFLD patients nationwide. The prevalence of NAFLD in junior high school students was also estimated as approximately 4% in certain areas of Japan

(Tsuruta et al., 2010). NAFLD is associated with obesity, insulin resistance, diabetes, hypertension, dyslipidemia, atherosclerosis, and systemic inflammation, representing hepatic manifestation of metabolic syndrome. Although it remains controversial whether NAFLD is a cause or a result of glucose intolerance and insulin resistance, a prospective study demonstrated higher risk of diabetes and cardiovascular events in non-diabetic humans with NAFLD compared with those without NAFLD (Heianza et al., 2014). Additionally, liver with significant steatosis is more susceptible for hepatotoxicants and retards/impairs regeneration following partial hepatectomy (Kele et al., 2013). Therefore, NAFLD is considered as a detrimental condition necessitating appropriate therapeutic interventions.

Dr. Ludwig, a pathologist in Mayo Clinic, proposed a term non-alcoholic steatohepatitis (NASH) in 1980 (Ludwig, Viggiano, McGill, & Oh, 1980). He described 20 non-alcoholic patients having histological findings as compatible with ASH, such as fatty changes, focal necrosis, ballooned hepatocytes with Mallory-Denk bodies, lobular inflammation, and perisinusoidal/perivenular fibrosis. Clinically, most of these patients were obese and 25% had diabetes. At present, NAFLD is classified into two categories according to liver pathology: non-alcoholic fatty liver (NAFL, previously designated as simple steatosis) and NASH. NASH is de-fined by the presence of hepatocyte ballooning, lobular inflammation, and/or fibrosis in addition to macrovesicular steatosis, and NAFL is characterized as macrovesicular steatosis without ballooned hepatocytes (Hashimoto, Tokushige, & Ludwig, 2015). This pathology-based classification stems from the concept that NASH can progress into advanced liver fibrosis and the prognosis is poorer than that of NAFL and exhibited the clinical outcome different from NAFL. Indeed, in our NASH cases with obesity, diabetes, hypertension, and dyslipidemia, ballooned hepatocytes were detected in the initial biopsied samples, in which liver fibrosis apparently progressed in 5 years (Fig. 1). Matteoni et al. identified that the outcomes of cirrhosis and liver-related death were more frequent in NAFLD patients with ballooned hepatocytes than in those without ballooned hepatocytes (Matteoni et al., 1999). Others reported that the survival of NASH patients, but not NAFL patients, was significantly lower than an age- and sex-matched reference population (Ekstedt et al., 2006). Based on these findings, the notion that NASH is a serious and progressive type of NAFLD has generally been accepted. The diagnosis of NASH and evaluation of histological severity of NAFLD are performed by the pathological findings of the liver, but liver biopsy is somewhat invasive and costly. Additionally, sampling errors and differences in diagnostic accuracy between independent pathologists can sometimes be problematic. Therefore, less invasive and more accurate strategies to discriminate between NAFL and NASH and predict actual steatosis/inflammation/fibrosis instead of liver biopsy have been evaluated (Fujimori et al., 2016; Hatta et al., 2010; Kitabatake et al., 2017; Matsubara et al., 2012; Tanaka, Ichijo, et al., 2006; Tanaka, Tanaka, et al., 2006; Tanaka, Matsubara, Krausz, Patterson, & Gonzalez, 2012; Tsutsui et al., 2010). Recent studies demonstrated that the presence of fibrosis, but not hepatocyte ballooning, was a determinant of poor prognosis in NAFLD patients (Angulo et al., 2015; Loomba & Chalasani, 2015). Indeed, such a case of NAFLD with careful 27-year follow-up was examined (Nagaya et al., 2008). This patient was diagnosed as having NAFL at the first liver biopsy but gradually developed into cirrhosis and HCC over 20 years. This case teaches us that NAFL is not always benign. Additionally, HCC may occur from NAFL regardless of the absence of advanced fibrosis,

past HBV infection, and regular ethanol consumption (Kimura et al., 2017). Although key factors affecting clinical course and outcome of NAFLD and methods to predict fibrosis progression and HCC development have not been identified, attenuating steatosis, hepatic injury, and inflammation and inhibiting fibrosis progression are promising strategies to improve the prognosis of NAFLD/NASH patients.

Understanding NAFLD/NASH pathogenesis is mandatory for developing novel therapeutic intervention strategies. Insulin resistance and diabetes were reportedly associated with NAFLD with more advanced fibrosis, while impaired glucose metabolism and insulin signaling aggravated liver fibrogenesis and NAFLD, in turn, worsening diabetes and driving systemic inflammation. A 'two-hit' model has been proposed to explain why some, but not all, individuals with steatosis develop steatohepatitis (Day & James, 1998). Besides diabetes/insulin resistance, many other second-hit mechanisms, such as lipotoxicity due to saturated fatty acid (FA), free cholesterol, and ceramide, endotoxins from gut and gum, oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, and iron overload, have been extensively reviewed. Additionally, contribution of other organs to the development of NAFLD/NASH should be considered (Jiang, Xie, Li, et al., 2015; Jiang, Xie, Lv, et al., 2015; Tanaka et al., 2014). Recently, a term 'multiple-hit theory' has been widely accepted because of close interconnections among these hits (Buzzetti, Pinzani, & Tsochatzis, 2016).

1.3. Nuclear receptors as principal regulators of energy/nutrient homeostasis

More recently, the adverse outcome pathway framework has been used to contextualize the role of nuclear receptors in hepatosteatosis (Mellor, Steinmetz, & Cronin, 2016; Willett et al., 2014). In humans, there are 48 nuclear receptors categorized into 7 subfamilies designated as NR0-NR6 (Evans & Mangelsdorf, 2014) (Table 1). Nuclear receptors are ligand-activated transcription factors regulating the expression of several genes through direct modulation of the transcriptional activities and epigenetic changes. Notably, nuclear receptors in the NR1 subfamily are associated with energy/nutrient control, which may play an important role in the pathogenesis of fatty liver disease. These NR1 nuclear receptors are NR1C1-3: peroxisome proliferator-activated receptor (PPAR) α , β/δ , and γ , NR1H2-3: liver X receptor (LXR) α and β , NR1H4: farnesoid X receptor (FXR), NR1I2: pregnane X receptor (PXR), and NR1I3: constitutive androstane receptor (CAR) (Table 1). These nuclear receptors are mainly activated by binding with ligands, form a heterodimer with retinoid X receptor (RXR) α , β , and γ (NR2B1-3), and exhibit their function as transcription factors. The ligands of NR1 subfamily include nuclear pore-permeable lipophilic endogenous substances mainly derived from nutrients [e.g., FAs, eicosanoids, oxysterols, and bile acids (BAs)] and exogenous chemicals. When the ligands are unbound, the activity of NR1 receptors is suppressed by binding to co-repressors. When either NR1 nuclear receptor or its heterodimeric partner RXR is liganded, these receptors release corepressors and recruit co-activators. However, the transcriptional signal is amplified when both heterodimeric receptors are liganded. The boosting effect of liganded RXR allows these NR1 receptors to significantly increase their transcriptional activities. Not only the ligands of RXR, but also the presence of RXR itself are important for the function of NR1 receptors. Indeed, loss of hepatocyte-specific RXR α disrupts the basal functions of NR1 receptors and alters nutrient metabolism (Anderson et al., 2004).

To understand the physiological role of NR1 nuclear receptors, the concept of “energy vector” was proposed (Evans & Mangelsdorf, 2014; Fig. 2). In the fasting state, triacylglycerol (TAG) stored in white adipose tissue (WAT) is subjected to lipolysis and released into the circulation as FAs. FAs are used in many organs as an energy source. In the liver, FAs activate PPAR α and enhance FA catabolism, resulting in the production of ATP, ketone bodies, and hepatokine fibroblast growth factor (FGF) 21. Ketone bodies are consumed as an energy source in the brain and FGF21 serves as a stress messenger to prepare other organs for energy deprivation. In the fed state, energy flux is reversed and FXR, LXR, PPAR β/δ and PPAR γ are mainly involved in nutrient absorption from the gut and distribution from gut/liver to peripheral tissues, such as WAT and muscle. After meals, BAs activate intestinal FXR, promoting nutrient absorption and maintaining a barrier to the gut microbiome. Absorbed dietary lipids are transported into the circulation as chylomicron and its remnant. Hepatic FXR promotes post-prandial TAG-rich lipoprotein clearance. Excess cholesterol is removed from the body by reverse cholesterol transport under the control of the FXR-stimulated enterokine FGF19 (FGF15 in rodents) and/or activation of hepatic LXR by oxysterols. Fecal elimination of cholesterol is the last step in the reverse cholesterol transport pathway. FGF19 increases hydrophilicity of the bile salt pool and stimulates transintestinal cholesterol excretion (de Boer et al., 2017). FGF15/19 also attenuates post-prandial hyperglycemia through enhancing hepatic glycogenesis. Consequently, excess nutrients are either consumed in muscle or stored in WAT due to PPAR β/δ and PPAR γ , respectively. Post-prandial hepatic activation of PXR and CAR promotes the clearance of toxic dietary metabolites and xenobiotics. Because abnormal energy/nutrient homeostasis is a major cause of NAFLD/NASH, the concept of “dysfunction of energy vectors on the gut-liver-adipose axis” may represent a mechanism on how dysregulated nuclear receptors contributes to NAFLD/NASH development (Fig. 2).

The following sections describe the roles of nuclear receptors, mainly focusing on NR1 receptors, in liver pathophysiology and possible therapeutic strategies for the prevention and treatment of fatty liver disease through targeting these key receptors.

2. PPAR α

2.1. PPAR overview

Since the administration of certain chemicals, such as Wy-14643, nafenopin, and fibrate derivatives, to mice induces hepatic peroxisome proliferation (increased peroxisome number and size) and hepatomegaly, these chemicals are called as peroxisome proliferators (PP) (Reddy & Krishnakantha, 1975). Furthermore, long-term PP administration results in HCC without accompanying hepatic fibrosis/inflammation (Reddy, Azarnoff, & Hignite, 1980). Since no apparent genetic mutations have been detected in this process, PPs have been recognized as nongenotoxic hepatocarcinogens (Reddy & Lalwai, 1983). To explain a mechanism of rapid and drastic changes following PP administration, the involvement of transcription factors was assumed and the first PPAR [later defined as PPAR α (NR1C1)] was identified in 1990 (Issemann & Green, 1990). Subsequently, two other PPARs, PPAR β/δ (NR1C2) and PPAR γ (NR1C3), were discovered (Kliwer et al., 1994). A mouse line lacking the PPAR α was established (Lee et al., 1995), demonstrating not only a crucial

role of PPAR α for the occurrence of peroxi-some proliferation, hepatomegaly, and HCC following PP treatment, but also the physiological function of PPAR α in the body (Boverhof et al., 2011).

The main characteristics of three PPARs are summarized in Table 2. The localization of PPARs is quite different. PPAR α is expressed in hepatocytes, cardiomyocytes, proximal renal tubular cells, and brown adipocytes. The expression of PPAR β/δ is more ubiquitous but mainly found in muscle, skin, adipose tissue, and liver. PPAR γ has three splicing variant isoforms (γ 1, γ 2, and γ 3) that display differences in tissue localization for each isoform; γ 1 (ubiquitous localization), γ 2 (localized in adipose tissue), and γ 3 (localized in macrophages, colon, and adipose tissue). The expression of PPAR α and PPAR γ is abundant in FA-consuming and FA-storing tissues, respectively.

The optimal response elements for PPARs are direct repeats of AGGTCA separated by a single nucleotide (AGGTCA-n-AGGTCA), termed direct repeat (DR) 1. Long-chain FAs and eicosanoids are typical endogenous ligands for PPAR α and PPAR β/δ (Narala et al., 2010; Yu et al., 1995), while PPAR γ is specifically activated by arachidonic acid metabolites belonging to 5-hydroxyeicosatetraenoic acid family, such as 5-oxo-15(*S*)-hydroxyeicosatetraenoic acid and 5-oxo-eicosatetraenoic acid (Altmann et al., 2007; O'Flaherty et al., 2005). Many synthetic PP agents, including lipid-lowering fibrates, exert PPAR α -activating properties in rodents. Several environmental toxicants and endocrine disruptors, such as pesticides (diclofap-methyl, pyrethins, and imazalil), herbicides (2,4-dichlorophenoxyacetic acid), phthalate esters (diethylhexyl phthalate), and aldehyde metabolites of chlorinated solvents (perchloroethylene and trichloroethylene), can activate PPAR α (Corton et al., 2014; Takeuchi, Matsuda, Kobayashi, Takahashi, & Kojima, 2006). However, existence of significant species-specific differences concerns the effects of these PPAR α activators (Gonzalez & Shah, 2008). Wy-14643 is a potent PPAR α activator with weak PPAR β/δ and PPAR γ activation potential (Kliwer et al., 1994). GW501516 is a highly-selective PPAR β/δ ligand, and anti-diabetic thiazolidinedione (TZD) derivatives, such as troglitazone, pioglitazone, GW1929, and GW2090 are specific PPAR γ activators.

Typical PPAR α target genes are those encoding FA β -oxidation enzymes (Mandard, Müller, & Kersten, 2004). When PPAR α is activated by FA, FA β -oxidation and ensuing ATP production and ketogenesis are enhanced, rendering PPAR α as the main FA utilizer and energy generator under nutrient-deprived state. On the contrary, when PPAR γ is activated, several proteins, such as adipocyte-specific FA-binding protein (FABP4, also known as aP2), are up-regulated and FAs are stored in adipocytes as TAG. Thus, different features of PPARs in cell-specific expression, ligands, and target genes clearly demonstrate a major but distinct contributions of the three PPARs to energy/nutrient homeostasis (Fig. 2).

2.2. Hepatic steatosis and PPAR α

Early studies revealed that constitutive mitochondrial β -oxidation activity was markedly reduced in Ppara-null mouse livers (~40% of wild-type mice) (Aoyama et al., 1998). Typical PPAR α target genes include carnitine palmitoyl-CoA transferase 1 α (CPT1A), carnitine-acylcarnitine translocase (SLC25A20), and medium-chain acyl-CoA dehydrogenase (ACADM) (Fig. 3). Since ACADM is a rate-limiting enzyme of mitochondrial FA β -

oxidation, and acyl-CoA oxidase 1 (ACOX1), a rate-limiting enzyme of peroxisomal FA β -oxidation (not shown in Fig. 3), is also the target of PPAR α , this finding revealed a crucial role for PPAR α in FA catabolism and clearance. Under the circumstance in which FAs are predominantly utilized as an energy source, such as fasting, *Ppara*-null mice cannot augment hepatic FA catabolism in response to increased FA influx, leading to severe steatosis (Hashimoto et al., 2000; Kersten et al., 1999).

In addition to the direct transcriptional activation of genes encoding FA-metabolizing enzymes, the control of energy and nutrient homeostasis via FGF21 is also important means of control. FGF21 is a hepatokine secreted from the liver into blood, binds to a plasma membrane receptor complex on target tissues, mainly the FGF receptor 1 and β -Klotho heterodimer, and enhances expression of glucose transporter 1 in extra-hepatic tissues, leading to improvement of systemic insulin sensitivity and lipid turnover (Kharitonov & Adams, 2013; Nishimura, Nakatake, Konishi, & Itoh, 2000). Recombinant FGF21 injection stimulates hepatic FA utilization and attenuates hepatic steatosis in mice, markedly decreases plasma glucose and lipid profiles in diabetic rhesus monkeys (Kharitonov et al., 2005, 2007), and improves serum lipid profiles in obese humans with type 2 diabetes (Gaich et al., 2013). Since mice lacking FGF21 gene (*Fgf21*) are prone to hepatosteatosis and decreased FA catabolism by treatment with a methionine- and choline-deficient (MCD) diet (Tanaka, Takahashi, Zhang, et al., 2015), FGF21 modulates nutrient metabolism to improve hepatic steatosis. Hepatic and serum levels of FGF21 are significantly increased by PPAR α activation, while the constitutive levels are markedly lower in *Ppara*-null mice compared with wild-type mice (Inagaki et al., 2007; Tanaka, Takahashi, Zhang, et al., 2015). These findings suggest that PPAR α action for hepatic lipid metabolism is partially mediated by FGF21 (Fig. 4).

Since PPAR α is also expressed at the low levels in gut and adipocytes, the contribution of hepatocyte PPAR α to whole-body metabolism has not been fully clarified using *Ppara*-null mice. A hepatocyte-specific *Ppara*-null mouse line was generated, which exhibited neither induction of hepatocyte proliferation following PP administration nor increases in plasma ketone bodies and FGF21 in a fasting state, similarly to *Ppara*-null mice (Brocker et al., 2017; Montagner et al., 2016). Additionally, mRNA levels of mitochondrial FA β -oxidation enzymes were basally low in these mouse livers and hepatic TAG was easily accumulated by fasting, aging, and MCD diet (Montagner et al., 2016). These findings corroborate the importance of hepatocyte PPAR α in steatogenesis.

2.3. Hepatic inflammation, fibrosis and PPAR α .

PPAR α also has anti-inflammatory properties through counteracting nuclear factor kappa B (NF- κ B) at the protein level (Staels et al., 1998). NF- κ B is a principal transcription factor regulating inflammatory signaling and its activation induces the expression of genes encoding proinflammatory mediators, such as tumor necrosis factor α (TNF α) and monocyte chemoattractant protein 1 (MCP1). PPAR α activation also up-regulates the expression of an inhibitor of NF- κ B, resulting in suppression of NF- κ B activity. Activation of c-Jun or p65 and their nuclear translocation are also prevented by PPAR α . (Delerive et al., 1999; Delerive, Gervois, Fruchart, & Staels, 2000; Delerive et al., 2002). Additionally, it is

noteworthy that FGF21 induced by PPAR α activation has hepatoprotective activity. Acetaminophen overdose-induced liver injury and mortality were significantly aggravated in *Fgf21*-null mice compared with wild-type mice, likely due to impaired PPAR γ coactivator 1 α (PGC1 α)-nuclear factor erythroid 2-related factor 2 (NRF2)-mediated antioxidant capacity and increased oxidative stress, and acetaminophen-induced hepatotoxicity in *Fgf21*-null mice were reversed by recombinant FGF21 supplementation (Ye et al., 2014).

However, the anti-inflammatory actions of PPAR α may be organ-specific and disease-specific. For example, in mice, PPAR α activation by fibrates is protective for acute liver injury (Fang et al., 2017; Patterson, Shah, Matsubara, Krausz, & Gonzalez, 2012), while it is harmful for inflammatory bowel disease (IBD) induced by dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzenesulfonic acid (Qi et al., 2014). Additionally, PPAR α activation increases the expression of the peroxisomal β -oxidation enzyme ACOX1, which generates hydrogen peroxide as a byproduct (Kim, Qu, et al., 2014). Therefore, persistent and potent PPAR α activation might be harmful for a certain type of cells or tissues.

Attenuated hepatic injury/inflammation by PPAR α activation may inhibit fibrosis progression. Indeed, Wy-14643 and fenofibrate attenuated thioacetamide-induced liver fibrosis in rats, which may be associated with the increased expression of catalase, reducing oxidative stress (Toyama et al., 2004). Recently, oleoylethanolamide, an endocannabinoid-like endogenous molecule which binds to PPAR α with high affinity, ameliorated thioacetamide-induced hepatic fibrosis by blocking activation of hepatic stellate cells (HSCs) and overexpression of α -smooth muscle actin (α SMA) and collagen matrix in a PPAR α -dependent manner (Chen et al., 2015). *In vitro* studies showed that oleoylethanolamide inhibited transforming growth factor β 1-stimulated HSC activation through suppressing SMAD2/3 phosphorylation, but this effect disappeared by co-treatment with the PPAR α antagonist GW6571 (Chen et al., 2015). These findings indicate the possibility that PPAR α ligands have anti-fibrotic properties.

2.4. Hepatic cancer and PPAR α

Administering PPAR α ligands to mice induces the expression of cell cycle-regulators and oncogenes, such as cyclin D1/E, cyclin dependent kinase 2/4, and c-Myc in a PPAR α -dependent manner (Peters et al., 1998). Prolonged and strong PPAR α activation leads to HCC in mice and rats (Furukawa, Numoto, Furuya, Furukawa, & Williams, 1985; Tanaka, Moriya, Kiyosawa, Koike, Gonzalez, et al., 2008; Tanaka, Moriya, Kiyosawa, Koike, & Aoyama, 2008), but not in humans. This phenomenon is mainly due to negative regulation of let-7C, a microRNA important in cell growth, resulting in enhanced expression of c-Myc and the oncogenic mir-17–92 cluster (Qu et al., 2014; Shah, Morimura, et al., 2007). Wy-14643 treatment to mice carrying human *PPARA* gene enhanced FA metabolism, but neither inhibited let-7C nor induced c-Myc/mir-17 expression and HCC. Based on these findings, let-7C down-regulation and ensuing inductions of c-Myc and mir-17 is one of the mechanisms of murine PP-induced hepatocarcinogenesis (Gonzalez & Shah, 2008). It remains unclear why such species differences exist in the mechanism on how PPAR α affects the expression of let-7C and its downstream targets, but the fact that PPAR α protein levels

are lower in human liver as compared with rodent liver is presumably linked with species differences in responsiveness to PP.

2.5. Targeting PPAR α for fatty liver disease

The beneficial effects of PPAR α for NAFLD/NASH have been proven in several mouse models by the treatments of high-fat diet, trans-fat-rich diet, and MCD diet (Hu et al., 2017). Using liver tissues from NAFLD/NASH patients, PPAR α expression was documented to become low with fibrosis progression (Francque et al., 2015; Nagaya et al., 2010). Considering several hepatoprotective functions of PPAR α , therapeutic strategies to restore PPAR α expression/activity may be beneficial. FGF21 induction by PPAR α activation may also improve insulin sensitivity and attenuate the activity of NAFLD/NASH. Although fibrates have been regarded as available PPAR α activators in humans, these agents have not been of clinical benefit in the treatment of NAFLD/NASH. For example, sixteen NASH patients with hypertriglyceridemia were treated with clofibrate 2 g/day for 12 months, but no changes from baseline were found in serum levels of alanine aminotransferase (ALT), TAG, and cholesterol, or in histological grade of steatosis, inflammation, or fibrosis after 12 months of treatment (Laurin et al., 1996). In another study, 48-week treatment with fenofibrate 200 mg/day to sixteen NAFLD patients reduced serum glucose, insulin, and TAG levels and the ratio of the patients having ALT > 45 IU/L, but did not yield any effects on the grade of steatosis, lobular inflammation, fibrosis, or NAFLD activity score (Fernández-Miranda et al., 2008). One possible explanation for these disappointing results is that the degree of PPAR α activation is relatively lower in humans as compared with rodents. When clinically-relevant dose of bezafibrate (200–400 mg/day) was administered to humans, the maximum concentration and area under the pharmacokinetic curve were 5–10 $\mu\text{g/mL}$ and 5–40 $\mu\text{g h/mL}$, respectively (Nakajima et al., 2009). To get similar pharmacokinetic values in mice, the suitable dose of bezafibrate is 10–30 mg/kg/day which does not activate murine PPAR α . Therefore, the clinical dose of fibrates might be insufficient to activate PPAR α and improve NAFLD/NASH in humans.

Tamoxifen is an estrogen receptor antagonist used for breast cancer treatment, but may cause NAFLD/NASH. Occurrence of liver dysfunction due to tamoxifen-induced NASH may become an obstacle for breast cancer patients. Bezafibrate attenuated hepatosteatorosis in spite of continuing tamoxifen intake (Saibara, Onishi, Ogawa, Yoshida, & Enzan, 1999); however, further investigations are needed to determine whether such beneficial effects are actually derived from PPAR α activation since bezafibrate can activate PPAR α and PPAR β/δ in mice (Peters, Aoyama, Burns, & Gonzalez, 2003).

When a 4% ethanol-containing Lieber-DeCarli diet was given to wild-type and *Ppara*-null mice, wild-type mice showed only steatosis, but the *Ppara*-null exhibited the pathological features resembling human ASH, increased oxidative stress, and NF- κ B activation (Nakajima et al., 2004). These ethanol-induced abnormalities were attenuated by co-administration of polyenephosphatidylcholine, a naturally-existing antioxidant, suggesting a key role of oxidative stress in the pathogenesis of ASH in *Ppara*-null mice (Okiyama et al., 2009). Since ethanol reduces PPAR α , therapeutic strategies to correct PPAR α expression and activity might be useful for the treatment of ALD and ASH.

Urea cycle disorders, such as carbamoyl-phosphate synthase deficiency and ornithine transcarbamoylase deficiency, are often accompanied by diffuse microvesicular steatosis mimicking Reye's syndrome, but a possible link between urea cycle disorders and disruption of hepatic lipid metabolism remains unclear. SLC25A13 (citrin or aspartate-glutamate carrier 2) is located in the mitochondrial membrane in the liver and its genetic deficiency causes adult-onset type II citrullinemia (CTLN2) (Kobayashi et al., 1999). CTLN2 is one of the urea cycle disorders characterized by sudden-onset hyperammonemia due to reduced argininosuccinate synthase activity and is frequently accompanied by hepatosteatosis in the absence of obesity and ethanol consumption (Komatsu et al., 2008; Saheki et al., 2002; Tanaka, Yazaki, & Kobayashi, 2007). When the expression of genes involved in lipid metabolism was examined using liver samples obtained from CTLN2 patients, the expression of enzymes involved in FA oxidation and PPAR α was markedly suppressed in CTLN2 livers (Komatsu et al., 2015). Hypoketonemia in these patients corroborated reduced mitochondrial β -oxidation activity, and hepatic PPAR α expression was inversely correlated with the severity of steatosis and circulating ammonia and citrulline levels. These findings provide a novel link between urea cycle disorder, lipid metabolism, and PPAR α , and suggest the possibility that modulation of PPAR α might have impacts on impaired urea and ammonia metabolism.

Collectively, PPAR α is an intriguing therapeutic target for fatty liver disease; novel agents to efficiently and safely stimulate human PPAR α are desired.

3. PPAR β/δ

3.1. Hepatic steatosis and PPAR β/δ

PPAR β/δ is highly expressed in muscle, skin, adipose tissue, and liver. PPAR β/δ levels are far higher in muscle than the other two forms of PPAR in rodents and humans and its activation during the fed state or exercise increases fuel consumption in muscle mainly via enhancement of β -oxidation (Manickam & Wahli, 2017). In obese monkeys, the treatment with PPAR β/δ agonist GW501516 normalizes serum insulin and TAG concentrations, increases high-density lipoprotein (HDL) cholesterol, and decreases low-density lipoprotein cholesterol levels (Oliver et al., 2001). Increased insulin sensitivity and decreased adiposity by PPAR β/δ activation were observed in diet-induced and genetically obese mice (Luquet et al., 2005). Mechanistically, PPAR β/δ activation increases hepatic carbohydrate catabolism by up-regulating glucose-6-phosphate dehydrogenase activity, suppresses hepatic glucose output, inhibits FA release from WAT, and promotes β -oxidation in muscle. Consistently, mice lacking PPAR β/δ gene (*Ppard*) are metabolically less active and glucose-intolerant (Lee et al., 2006). Although PPAR β/δ might attenuate hepatosteatosis under insulin-resistant state (Wang et al., 2003), the contribution of PPAR β/δ to hepatic lipid metabolism is controversial. Intravenous injection of adenovirus carrying the PPAR β/δ cDNA to *db/db* mice induced hepatic expression of the insulin-induced gene-1 and suppressed sterol-regulatory element-binding protein (SREBP) 1 activation that up-regulates lipogenic enzymes (showing blue in Fig. 3) and drives *de novo* lipogenesis in the hepatocytes, thus ameliorating hepatosteatosis (Qin et al., 2008). However, treatment of *db/db* mice with GW501516, a high-affinity PPAR β/δ ligand, enhanced expression of a lipogenic enzyme

acetyl-CoA carboxylase β (ACACB) and increased hepatic TAG contents (Lee et al., 2006). Others reported that GW501516 did not improve hepatosteatosis in high-fat diet-fed mice despite enhancement of the PPAR α -PGC1 α axis and adenosine monophosphate-activated protein kinase (AMPK) phosphorylation (Barroso et al., 2011). Therefore, the direct impact of PPAR β/δ on hepatic lipid metabolism is presently elusive.

3.2. Hepatic inflammation, fibrosis, cancer, and PPAR β/δ

Ppard-null mice are more susceptible to carbon tetrachloride (CCl₄)-induced hepatotoxicity, and the highly specific PPAR β/δ ligand GW0742 attenuates hepatotoxicity in a PPAR β/δ -dependent manner likely through attenuating NF- κ B signaling (Shan, Nicol, et al., 2008). Primary hepatocytes and HSC from *Ppard*-null mice exhibited higher TNF α and α SMA expression, respectively, compared with wild-type mice, but GW0742 treatment did not affect inflammatory signaling in hepatocytes and HSC activation (Shan, Palkar, et al., 2008), suggesting that immune cells, such as Kupffer cells, are responsible for anti-inflammatory effects of PPAR β/δ activators. This view is partially supported by microarray data revealing that various inflammation-related processes, including antigen presentation and natural killer cell-mediated cytotoxicity, were up-regulated in *Ppard*-null mouse livers (Shan, Nicol, et al., 2008).

On the other hand, the effect of PPAR β/δ activation on liver fibrosis remains controversial. A synthetic PPAR β/δ ligand KD3010 attenuated liver fibrosis induced by CCl₄ and bile duct ligation in mice (Iwaisako et al., 2012), but GW501516 promoted it (Kostadinova et al., 2012). Additionally, the relationship between HCC and PPAR β/δ activation has not been documented.

3.3. Targeting PPAR β/δ for fatty liver disease

Although there are limited data on the possible protective role of PPAR β/δ on liver fibrosis and hepatocarcinogenesis, PPAR β/δ might attenuate fatty liver disease through improving insulin sensitivity in muscle and suppressing inflammatory signaling. GW501516 attenuated NASH in MCD diet-treated mice by down-regulating TNF α and MCP1 expression (Nagasawa et al., 2006). Considering weak effects of PPAR β/δ activators on hepatic steatosis and fibrosis, a dual PPAR α/β activator, elafibranor, has been developed which showed beneficial effects for human NASH (Ratziu et al., 2016). In this GOLDEN 505 trial, patients with NASH without cirrhosis were randomly assigned to groups given elafibranor 80 mg (n = 93), elafibranor 120 mg (n = 91), or placebo (n = 92) each day for 52 weeks. NASH resolved without exacerbation of fibrosis in a higher proportion of patients in the 120-mg elafibranor group vs the placebo group (19% vs 12%; P = 0.045). Patients with NASH resolution after receiving 120-mg elafibranor had reduced fibrosis stages compared with those without NASH resolution (mean reduction of 0.65 \pm 0.61 in responders for the primary outcome vs an increase of 0.10 \pm 0.98 in non-responders; P < 0.001). Elafibranor was well tolerated and did not cause weight gain or cardiac events, but did produce a mild, reversible increase in serum creatinine.

Evidence on the association between ALD/ASH and PPAR β/δ is also limited. A cohort of 4% ethanol diet-fed *Ppard*-null mice exhibited steatosis likely due to increased SREBP1c

activity (Goudarzi et al., 2013), while it remains unclear whether PPAR β/δ modulates ethanol-induced oxidative stress, abnormalities in immune responses, and HSC activation. Further studies are needed to address these issues and consider applications of PPAR β/δ activators to humans.

4. PPAR γ

4.1. Hepatic steatosis and PPAR γ

PPAR γ is highly expressed in adipose tissue and macrophage where it has an important role in energy storage and immune modulation, respectively (Ahmadian et al., 2013). PPAR γ expression in hepatocytes is relatively low, but is increased in human steatotic livers and mouse NAFLD/NASH livers. Forced expression of PPAR γ in hepatocytes by adenovirus induced hepatosteatosis, and hepatocyte-specific disruption of PPAR γ in *ob/ob* mice attenuated fatty liver (Matsusue et al., 2003; Yu et al., 2003). PPAR γ up-regulates the expression of genes involved in adipogenesis, such as FABP4 and cell death inducing DFFA like effector c [fat-specific protein 27 (FSP27)], a key protein coating lipid droplet in the cells, and hepatic FSP27 expression is associated with the development of fatty liver disease (Matsusue et al., 2008; Tanaka, Takahashi, Matsubara, et al., 2015; Tanaka, Takahashi, Zhang, et al., 2015; Xu, Cai, et al., 2015; Xu, Park, et al., 2015). Therefore, PPAR γ -mediated fatty liver is sometimes called as “adipogenic steatosis” (Yu et al., 2003). It remains unclear how PPAR γ is ectopically expressed and its expression is regulated in the liver. Hepatic PPAR γ expression is down-regulated by hairy and enhancer of split 6 (Hes6), a basic helix-loop-helix transcription repressor (Martinez-Jimenez, Kyrmizi, Cardot, Gonzalez, & Talianidis, 2010), while the promoter of *Hes6* gene is activated by retinoic acid receptor (RAR) α (NR1B1). Thus, all-trans retinoic acid, a typical RAR α ligand, ameliorated hepatic steatosis in obese mouse models through the RAR α -Hes6 axis activation and resulting PPAR γ 2 down-regulation (Kim, Kim, et al., 2014). Additionally, a novel mechanism that PPAR γ expression is negatively regulated by Fos-related antigen 1 (Fra-1) and Fra-2 was recently proposed (Hasenfuss et al., 2014). High-fat diet suppressed hepatic Fra-1 expression causing PPAR γ -mediated steatosis, while hepatic Fra-1 overexpression down-regulated PPAR γ and reduced TAG accumulation.

While hepatic overexpression/activation of PPAR γ is steatogenic, treatment of genetically-obese or diet-induced NAFLD/NASH mice with PPAR γ ligands reduces hepatic TAG contents. This discrepant effect of PPAR γ is mainly due to adiponectin, an adipokine discovered in 1995 (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995). Circulating adiponectin enhances glucose uptake in peripheral tissues and FA β -oxidation in hepatocytes through activation of AMPK, attenuating hepatic steatosis and improving systemic insulin sensitivity (Ye & Scherer, 2013). Additionally, adiponectin has cytoprotective and anti-cancer effects with yet fully unidentified mechanisms (Ye & Scherer, 2013). Indeed, hypoadiponectinemia is detected in rodents/humans with obesity, diabetes, and/or NAFLD/NASH, and adiponectin attenuates NASH (Fukushima et al., 2009; Hui et al., 2004; Kamada et al., 2007). Since PPAR γ activators are potent inducer of adiponectin in adipose tissues, these agents exert anti-steatotic and hepatoprotective effects mainly by increasing adiponectin expression.

Up-regulation of FGF21 is another mechanism for the anti-steatotic effects of PPAR γ , as well as PPAR α . Expression of FGF21 is significantly increased by rosiglitazone and pioglitazone in mouse livers and primary hepatocytes in a PPAR α -independent manner (Oishi & Tomita, 2011).

4.2. Hepatic inflammation, fibrosis, cancer and PPAR γ

Similar to other PPAR isoforms, PPAR γ attenuates inflammation by suppressing NF- κ B activity and increasing circulating anti-inflammatory mediators, such as adiponectin and FGF21. PPAR γ activation inhibits the production of TNF α and interleukin (IL) 1 β in monocytes and macrophages and primes monocytes into anti-inflammatory M2 macrophages (Bouhlef et al., 2007). In agreement with these findings, macrophage-specific disruption of PPAR γ gene (*Pparg*) in mice resulted in higher levels of necroinflammation/fibrosis, lipid peroxidation, caspase activation, and pro-inflammatory cytokine expression following repeated CCl₄ administration compared with hepatocyte-specific *Pparg*-null and wild-type mice (Morán-Salvador et al., 2013).

Similar exacerbation of hepatic necroinflammation/fibrosis was reported in HSC-specific *Pparg*-null mice (Morán-Salvador et al., 2013). However, this mouse line used the Cre-LoxP system and Cre recombinase expression under control of the *Fabp4* promoter. Since mouse FABP4 is also expressed in adipocytes, this result cannot exclude the influence of adipose PPAR γ disruption. PPAR γ , predominantly PPAR γ 2, is highly expressed in quiescent HSC compared as hepatocytes and Kupffer cells, and PPAR γ is down-regulated during HSC activation (Miyahara et al., 2000). PPAR γ agonists inhibit HSC proliferation and drive activated HSC to apoptosis and the quiescent phenotype, leading to attenuation of liver fibrosis (Bae et al., 2010). However, clinical application of anti-fibrotic effect of PPAR γ agonist remains inconclusive. In a trial of an experimental TZD, farglitazar, no efficacy was found on HSC activation or fibrosis in chronic hepatitis C patients (McHutchison et al., 2010).

Several studies using HCC cell lines demonstrated that rosiglitazone/ pioglitazone induced cell cycle arrest and apoptosis in a PPAR γ -dependent and -independent mechanism. Mice with heterozygous *Pparg* disruption were more susceptible to diethylnitrosamine-induced HCC and rosiglitazone administration reduced the incidence of HCC (Yu et al., 2010). These findings imply anti-cancer properties of PPAR γ activators.

4.3. Targeting PPAR γ for fatty liver disease

The beneficial effect of PPAR γ activators has been proven in mouse NAFLD/NASH model. The usefulness of rosiglitazone for human NASH was evaluated in the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) trial in 2008 (Ratziu et al., 2008). Rosiglitazone improved steatosis in correlation with reduced serum ALT and increased insulin sensitivity and adiponectin levels, but with no improvement in other histologic lesions, including fibrosis and NAFLD activity score. In paired biopsied samples from the participants in the FLIRT trial, rosiglitazone treatment raised hepatic expression of MCP1 compared with the baseline (Lemoine, Serfaty, Cervera, Capeau, & Ratziu, 2014). In the Pioglitazone versus Vitamin E versus Placebo for the Treatment of

Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, NASH patients treated with pioglitazone had dramatically improved steatosis and lobular inflammation, while hepatocellular ballooning and fibrosis were not improved (Sanyal et al., 2010). In a recent randomized double-blinded placebo-controlled trial for pre-diabetic or diabetic NASH patients, 18-month pioglitazone treatment achieved higher NASH histological improvement rate and resolution rate with statistically significant improvement in steatosis, inflammation and ballooning, as well as correction of insulin resistance and hypoadiponectinemia, compared with placebo (Cusi et al., 2016). Pioglitazone reduced the prevalence of NASH patients showing fibrosis progression compared with placebo, but extending the treatment to 36 months did not regress the fibrosis. Collectively, pioglitazone may be beneficial for diabetic NASH patients with none-to-mild liver fibrosis, but it remains unclear whether this agent can reverse advanced fibrosis and improve the outcome in cirrhotic NASH patients.

The efficacy and safety of PPAR γ agonists for human ALD/ASH has not been reported. In rats fed an ethanol-containing liquid diet for 6 weeks, pioglitazone significantly attenuated steatosis and lipid peroxidation without altering insulin resistance, likely due to restoring c-Met down-regulation (Tomita et al., 2004). This agent also lowered sensitivity against lipopolysaccharides in Kupffer cells and moderated necroinflammation (Enomoto et al., 2003). Future studies are necessary to assess the usefulness of PPAR γ agonists for ALD/ASH patients.

NAFLD sometimes develop after pancreaticoduodenectomy (PD). It was reported that the incidence of newly developed NAFLD following PD (*de novo* post-PD NAFLD) is 23–37% and some cases are histologically diagnosed as having NASH (Tanaka et al., 2011). Clinicians are concerned about such cases because these patients do not usually have obesity or metabolic syndrome (i.e., non-obese or lean NAFLD/NASH). NAFLD/NASH may be caused by malnutrition due to pancreatic exocrine insufficiency, because hepatic steatosis, body weight, hypoalbuminemia, and liver dysfunction were improved by intensifying pancreatic enzyme supplementation (Tanaka, Horiuchi, Yokoyama, Kawa, & Kiyosawa, 2008; Tanaka et al., 2011). When the expression of genes involved in lipid metabolism was examined using liver samples obtained from post-PD NAFLD/NASH patients, the mRNA levels of the genes encoding PPAR γ and its downstream CD36 and FABP4 were markedly enhanced. The gene expression pattern involved in hepatic lipid metabolism in post-PD NAFLD/NASH was different from fibrosis-matched conventional NAFLD/NASH livers, reflecting a distinct mechanism of steatogenesis (Nagaya et al., 2015). These findings provide a novel link among malnutrition, PPAR γ , and adipogenic steatosis. Indeed, hepatic steatosis and PPAR γ overexpression were detected in non-obese lipodystrophic mice (Tanaka, Takahashi, Matsubara, et al., 2015).

While TZD is a potent insulin sensitizer, several adverse effects, such as body weight gain, body fluid retention, and heart failure, temper the enthusiasm for use of this agent (Ahmadian et al., 2013). In the FLIRT study, weight gain was the main adverse effect (mean gain of 1.5 kg in the rosiglitazone group vs. –1 kg in the placebo group) and painful swollen legs was the main reason for dose reduction/discontinuation. Similar adverse effects were also noted in the PIVENS study. Two independent studies demonstrated that PPAR γ

activation in the brain contributed to the TZD-induced weight gain (Lu et al., 2011; Ryan et al., 2011). Fluid retention, peripheral edema, anasarca, and congestive heart failure are the major side effects of TZD as well. PPAR γ activation in renal distal collecting ducts was reported to alter water/sodium reabsorption ability, causing body fluid retention (Zhang et al., 2005). Further long-term TZD treatment may cause a reduction in bone mineral density and increase fracture risks in humans. A deleterious bone loss caused by PPAR γ agonists was prevented in *Fgf21*-null mice, and *Fgf21* transgenic mice and repeated recombinant FGF21-injected mice demonstrated marked trabecular bone loss (Wei et al., 2012). Mechanistically, FGF21 inhibited osteoblastogenesis and stimulated adipogenesis from bone marrow mesenchymal stem cells by potentiating PPAR γ activity. While the PPAR γ -FGF21 axis might be harmful for bone remodeling (Fig. 4), it remains elusive because of the presence of contradicting results (Li et al., 2017). Based on these observations, the development of new PPAR γ agonists with higher specificity to the target tissues/ cells (e.g., adipose-specific PPAR γ stimulator) might reduce the incidence of adverse effects while maintaining efficacy.

5. LXR

5.1. LXR overview

There are two LXR isoforms in humans, LXR α (NR1H3) and LXR β (NR1H2). LXR activates hepatic TAG synthesis and export to peripheral tissues, and stimulates reverse cholesterol transport to moderate cholesterol toxicity in extrahepatic tissues (Hong & Tontonoz, 2014). LXR α is highly expressed in the liver, intestine, kidney and adipose tissue, while LXR β is ubiquitously expressed (Shinar et al., 1994; Willy et al., 1995).

LXRs bind to DR4, direct repeats of the core sequence AGGTCA spaced by four nucleotides, which is called the LXR response element (Willy et al., 1995). Since the core of this element is identical to that of CAR and PXR, LXR target genes overlap with those of CAR and PXR to some degrees (Quack, Frank, & Carlberg, 2002). The typical endogenous ligands for LXR are oxysterols, which are generated through nonenzymatic auto-oxidation or enzymatic reaction (Gill, Chow, & Brown, 2008). In the former case, oxysterols tend to be ring hydroxylated, while in the enzymatic reaction, oxysterols are predominantly hydroxylated on the side chain to form a stronger activator of LXR, such as 24(*S*)-hydroxycholesterol, 24(*S*), 25-epoxycholesterol, and 22(*S*)-hydroxycholesterol. Additionally, there are several synthetic LXR ligands, such as GW3965 and T0901317; GW3965 is an LXR α / β dual agonist. T0901317 is a selective LXR α agonist, but is also a potent PXR agonist (Shenoy et al., 2004). Some environmental pollutants and endocrine disruptors, including bisphenol A, phthalates, and organophosphates, can interact with LXR α (Mozzicafreddo et al., 2015).

Typical target genes of LXR are those encoding FA synthase (FASN), acetyl-CoA carboxylase, and SREBP1c (Zhang et al., 2012; Zhong et al., 2013) The *SREBP1c* promoter contains two closely separated DR4 response elements (Fernández-Alvarez et al., 2011). Since SREBP1 regulates the transcriptional levels of genes encoding several lipogenic enzymes, such as ATP citrate lyase (ACLY), acetyl-CoA synthase, FASN, acetyl-CoA carboxylase α (ACACA), and ACACB (Fig. 3), its activation by LXR worsens hepatic TAG

accumulation. LXR also induces expression of the ATP-binding cassette transporters (ABC), such as ABCA1 and ABCG1 (Costet, Luo, Wang, & Tall, 2000; Kennedy et al., 2001), which mobilizes cholesterol from the peripheral tissue, enhances HDL formation, and attenuates atherosclerosis.

5.2. Hepatic steatosis and LXR

In humans, hepatic LXR expression was correlated with severity of NAFLD (Ahn, Jang, Jun, Lee, & Shin, 2014). Although LXR stimulation enhances SREBP1-mediated lipogenesis and worsens hepatosteatosis, LXR appears to improve diabetes by decreasing hepatic gluconeogenesis, increasing glucose-stimulated pancreatic insulin secretion, and upregulating adipose glucose transporters including glucose transporter type 4 (Ding et al., 2014). A recent observation revealed that some of the steatotic effects of LXR agonists are modulated in part by the IL-6 responsive gene product, CCAAT/enhancer-binding protein β , since mice lacking this gene exhibited lower levels of steatosis in response to LXR agonists (Rahman et al., 2013).

5.3. Hepatic inflammation, fibrosis, cancer and LXR

LXR tends to suppress inflammation, since SUMOylated LXR can inhibit NF- κ B activity (Venteclef et al., 2010). Another important anti-inflammatory mechanism may be derived from elevated expression of the *ABCA1* gene and cholesterol depletion. Cholesterol levels in the plasma membrane affect the function of Toll-like receptors (TLRs) and higher ABCA1 expression by LXR activation reduces plasma membrane cholesterol contents and sensitivity of TLRs (Zhu et al., 2010). However, a recent animal study showed a significant reduction in steatosis, inflammation, and collagen disposition by LXR antagonist, SR9238 in high-fat diet-induced NAFLD/NASH mice (Griffett et al., 2015). Relatively less is known about LXR and hepatocarcinogenesis.

5.4. Targeting LXR for fatty liver disease

LXR activation may suppress inflammation and improve atherosclerosis, but promote the development of obesity and hepatosteatosis. LXR antagonism may attenuate hepatosteatosis and ensuing fibrosis. Therefore, liver-specific LXR antagonist might be efficacious for fatty liver disease without giving any impact to reverse cholesterol transport system.

6. FXR

6.1. FXR overview

FXR was identified as a nuclear receptor activated by farnesol pyro-phosphate in 1995 (Forman et al., 1995). FXR exists as two variants in humans, FXR α (NR1H4) and FXR β (NR1H5), while the latter is a pseudogene (Evans & Mangelsdorf, 2014; Zhang, Cayting, Weinstock, & Gerstein, 2008). FXR is abundantly expressed in the organs involved in BA metabolism and transport, such as liver, intestine, and kidney, but also is present in adipose tissue and adrenal gland. Upon ligand activation, FXR binds to the transcriptional responsive elements as either monomers or heterodimers with RXR (Evans & Mangelsdorf, 2014). BAs are the principal FXR ligands (Makishima et al., 1999), while androsterone, anthracyclines, dihydropyridines, pyrethroids, and vinca alkaloids also activate FXR (Hsu et al., 2014).

Obeticholic acid (OCA, also known as INT-747), a 6 α -ethyl derivative of chenodeoxycholic acid (CDCA), is the first-in-class selective FXR agonist that is approximately 100-fold potent than CDCA for FXR activation (Pellicciari et al., 2002). Other synthetic agonists, such as GW4064, WAY-362450 (FXR-450 or XL335), and PX-102, have been widely used in animal experiments.

FXR serves as a sensor to regulate BA enterohepatic circulation. In the post-prandial state, intestinal FXR is activated by BAs (de Aguiar Vallim, Tarling, & Edwards, 2013), induces BA-binding protein in enterocytes, and facilitates BA/cholesterol absorption. Additionally, intestinal FXR activation increases the expression of FGF15/19, which is transported via portal circulation to the liver, where it binds to the FGF receptor 4/ β -klotho complex and suppresses the expression of cholesterol 7 α hydroxylase (CYP7A1), the rate-limiting enzyme of BA synthesis. When hepatocyte FXR is activated by BAs, it inhibits CYP7A1 expression through up-regulated small heterodimer partner (SHP, NR0B2) and increases the expression of ABCB11 (bile salt export pump, BSEP), a major BA transporter from hepatocytes to bile canaliculi and a typical FXR target. A recent study using human precision cut liver slices after OCA treatment revealed that *FGF19*, *NR0B2*, *ABCB11*, *SLC51A* [organic solute transporter α (OST α)], and *SLC51B* (OST β) are induced by OCA, corroborating a crucial role of FXR in hepatic BA metabolism in humans (Ijssennagger et al., 2016). FGF21 is also reported to be a transcriptional target of hepatic FXR (Cyphert et al., 2012), while the contribution of FXR to FGF21 induction is weaker than that of PPAR α and PPAR γ .

6.2. Hepatic steatosis and FXR

Several studies reported that hepatic FXR activation attenuated steatosis in rodents and humans and that *Fxr*-null mice are prone to hepatosteatois following high-fat diet (Li, Jadhav, & Zhang, 2013). Since few genes involved in FA/TAG metabolism are directly up-regulated by FXR activation, the mechanism of the anti-steatotic effect of FXR may be indirect, presumably through improvement of insulin resistance and lipoprotein transport.

The role of intestinal FXR in hepatic steatosis is controversial. Activation of FXR by a synthetic agonist, GW4064, strongly induced FGF15/19 expression in the intestine and further inhibited *Cyp7a1* gene expression leading to an inhibition of BA synthesis in the liver. Pharmacological administration of FGF15/19 increased the metabolic rate and reversed high fat-induced diabetes while decreasing adiposity (Fu et al., 2004). Intestine-specific FXR activation by fexaramine in mice reduced diet-induced weight gain, steatosis, and hepatic glucose production without activating FXR target genes in the liver, which was mediated via FGF15 signaling (Fang et al., 2015). However, down-regulation of intestinal FXR signaling either with an intestine-specific *Fxr*-null mice or pharmacologically with an FXR antagonist reduced circulating ceramide levels, inhibited hepatic SREBP1 signaling and attenuated hepatic steatosis (Gonzalez, Jiang, & Patterson, 2016; Jiang, Xie, Li, et al., 2015; Jiang, Xie, Lv, et al., 2015). Therefore, tissue-specific modulation of FXR signaling, e.g., liver-specific FXR agonist, intestine-specific FXR agonist or antagonist, can be of potential value for the treatment of metabolic diseases, including fatty liver and type 2 diabetes (Xie et al., 2017).

6.3. Hepatic inflammation, fibrosis, cancer and FXR

Intestinal FXR plays a crucial role in maintaining small intestine bacterial homeostasis and intestinal barrier function to protect against bacterial translocation (Wiest, Lawson, & Geuking, 2014) and to attenuate hepatic TLR activation. The effects of FXR in hepatic inflammation and fibrosis were demonstrated in mice lacking *Fxr*; and by activation with synthetic FXR agonists. *Fxr*-null mice fed a high-fat diet had bacterial overgrowth with increased intestinal permeability and higher levels of pro-inflammatory and pro-fibrogenic factors. FXR activation by GW4064 and CDCA promoted the expression of several genes that have antimicrobial properties, inhibited NF- κ B expression, and reduced pro-inflammatory cytokines (Inagaki et al., 2006). FXR activation by GW4064 improved hepatic inflammation and NASH induced by a high fat/high cholesterol diet (Ma, Huang, Yan, Gao, & Liu, 2013). Likewise, another synthetic FXR agonist, WAY-362450, attenuated MCD diet-induced NASH (Zhang, Wang, Liu, & Harnish, 2009).

Compelling evidence suggests that FXR may regulate HCC development. *Fxr*-null mice develop BA overload and spontaneous HCC by the age of 12–16 months (Kim et al., 2007; Yang et al., 2007). A recent study demonstrated that intestine-specific reactivation of FXR in *Fxr*-null mice relieved these mice from BA overload and prevented HCC development via the intestinal FXR-FGF15 axis control of BA homeostasis (Degirolamo et al., 2015). While this study suggests that intestinal FXR via the intestine FXR-FGF15 pathway protects against HCC induced by the absence of hepatic FXR, it is not physiologically relevant as there are no scenarios where FXR is deficient in liver in either mice or humans, other than the rare human FXR deficiencies that are lethal (Gomez-Ospina et al., 2016). However, further studies are needed to clarify the role of FXR in hepatocarcinogenesis.

6.4. Targeting FXR for fatty liver disease

Hepatic FXR expression is decreased in NAFLD patients (Yang, Shen, & Sun, 2010). Since FXR activation decreases hepatic lipogenesis, FXR activation may be a promising therapeutic target for NAFLD. In the more recently published study named “The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment” (FLINT) study, NASH patients were treated with OCA (Neuschwander-Tetri et al., 2015). While obesity, steatosis, inflammation, and fibrosis improved, circulating cholesterol levels, pruritus, and insulin sensitivity worsened. Hypercholesterolemia after OCA treatment may reflect FXR activation and ensuing CYP7A1 down-regulation, inhibiting cholesterol catabolism to BA. While hepatic insulin resistance worsened after OCA treatment, a small study in diabetic patients revealed the improvement of insulin responsiveness, as evidenced by hyperinsulinemic euglycemic clamp method. Aggravated insulin resistance might be an adaptive mechanism in response to long-term OCA treatment. However, FXR activation was also shown to exacerbate weight gain and glucose intolerance (Watanabe et al., 2011). The benefits of FXR activation by synthetic ligands require further investigation, and a phase III clinical trial of OCA (REGENERATE) is underway. Additionally, enhanced tissue specificity of FXR stimulation is an attractive strategy to improve clinical benefit.

While the efficacy of FXR agonists for ALD/ASH is limited, OCA reversed hepatic steatosis and oxidative stress induced by ethanol (Lívero et al., 2014). Thus, OCA may be a promising agent for ALD/ASH.

7. Other NR1 nuclear receptors and fatty liver disease

7.1. PXR

PXR (NR1I2) is a xenobiotic-sensing nuclear receptor that regulates drug metabolism and detoxification. PXR is abundant in the liver and gut, and is expressed not only in hepatocytes, but also in HSC and Kupffer cells (Banerjee, Robbins, & Chen, 2015). PXR recognizes DR3 and DR4 sequences as well as everted repeats separated by 6 or 8 base pairs (Kliwer, Goodwin, & Willson, 2002). A typical target gene of PXR is cytochrome P450 (CYP) 3A4, but the spectrum of PXR targets has expanded to genes involved in energy homeostasis, such as stearoyl-CoA desaturase 1, FA elongase, and the FA transporter CD36 (Wada, Gao, & Xie, 2009; Zhou et al., 2008). PXR also regulates other transcription factors involved in metabolic homeostasis, such as SREBP1 and PPAR α (Bitter et al., 2015; Li et al., 2016).

PXR-humanized mice exhibited obesity and glucose intolerance when compared to wild-type mice fed a high fat diet (Spruiell, Richardson, et al., 2014; Spruiell, Jones, et al., 2014). Long-term stimulation of humanized PXR by rifaximin, a non-absorbed antibiotics and PXR ligand in humans, caused hepatosteatorosis mainly by up-regulating intestinal FA-binding protein and CD36 expression (Cheng, Krausz, et al., 2012). A clinical study reported insulin resistance in volunteers treated with the PXR agonist rifampin (Rysä et al., 2013). Finally, at least two human NR1I2 polymorphisms, rs7643645/G and rs2461823, were reportedly associated with severe phenotype of NAFLD (Sookoian et al., 2010). Collectively, PXR activation worsens steatosis, obesity, and insulin resistance due to increased hepatic FA uptake and synthesis and decreased β -oxidation (Li et al., 2015; Zhou et al., 2006, 2008).

PXR activation is anti-inflammatory among many tissues through down-regulating NF- κ B target genes. Activation of mouse PXR with pregnenolone 16 α -carbonitrile was found to be protective against DSS-induced IBD while *Pxr*-null mice had exaggerated it (Shah, Ma, et al., 2007). Symptoms of DSS-induced IBD were also ameliorated in PXR-humanized mice treated with rifaximin (Cheng et al., 2010; Cheng, Shah, & Gonzalez, 2012). The effects of both mouse and human PXR are due in large part to suppression of NF- κ B and decreased inflammation (Cheng, Shah, et al., 2012). Rifaximin was proposed as a treatment for Crohn's disease (Sartor, 2016), and was approved for the treatment of irritable bowel syndrome, a milder form of IBD (Lucak, Chang, Halpert, & Harris, 2017). The mouse PXR agonist pregnenolone 16 α -carbonitrile also attenuated CCl₄-induced liver fibrosis in mice (Marek et al., 2005), but anti-fibrotic effect for NASH remains uninvestigated.

The role of PXR in hepatocarcinogenesis was addressed in HCC mediated by aflatoxin B1 (AFB1) and HBV, both often seen in Asian populations. AFB1, a procarcinogenic mycotoxin, activated PXR and increased CYP3A4 expression and the resultant production of carcinogenic epoxide (Ratajewski, Walczak-Drzewiecka, Sałkowska, & Dastyh, 2011). Additionally, HBV X protein interacts with the ligand binding domain of PXR and increases

the transcriptional activity of PXR at the CYP3A4 promoter (Niu et al., 2013). Therefore, the synergistic effects of AFB1 and HBV on hepatocarcinogenesis might be explained by PXR-dependent mechanisms. However, the role of PXR in hepatocarcinogenesis stemming from fatty liver disease requires further investigation.

7.2. CAR

CAR (NR1H3) is activated through ligand binding or post-transcriptional modifications, such as phosphorylation (Kobayashi, Hashimoto, Honkakoski, & Negishi, 2015; Mutoh et al., 2009). The well-characterized endogenous ligands of CAR include bilirubin, BAs, and androstanes, while phenobarbital and some pesticides are typical xenobiotic ligands. Human CAR is highly expressed in the liver and intestine with lower expression in the heart, muscle, kidneys, and lung. CAR transcriptionally regulates several genes encoding xenobiotics-metabolizing enzymes and drug transporters, including *CYP2B6*, uridine 5-diphospho-glucuronosyltransferase, sulfotransferase, and MDR1 (*ABCB1*) (Banerjee et al., 2015; Klaassen & Aleksunes, 2010).

CAR protects against toxic food contaminants or metabolites (Beilke et al., 2009; Uppal et al., 2005). Interestingly, CAR activity was observed to follow a circadian rhythm in mice. CAR is less active during the day, but during the night, when mice eat, CAR is highly active, indicating the interconnection between CAR-mediated xenobiotic metabolism and energy metabolism. Indeed, the expression of *CYP2B6* and *ABCG2*, typical CAR target genes was up-regulated with increasing NAFLD severity (Fisher et al., 2009; Hardwick, Fisher, Canet, Scheffer, & Cherrington, 2011) and this change was thought to be an adaptive response to metabolic stress. Treatment with the CAR agonist TCPOBOP attenuated diet-induced obesity and diabetes in animal models through reducing hepatic gluconeogenesis (Dong et al., 2009; Gao, He, Zhai, Wada, & Xie, 2009). Additionally, CAR activation improved hepatic steatosis by diminishing lipogenesis and inducing β -oxidation in a mouse model (Dong et al., 2009).

CAR has anti-inflammatory and anti-apoptotic properties in NAFLD, but its potential role in hepatic fibrosis and HCC are controversial. Administration of a CAR agonist reduced inflammation and hepatocellular apoptosis, but worsened hepatic fibrosis in MCD diet-fed NASH mice (Yamazaki et al., 2007). CAR activation promoted hepatocarcinogenesis and loss of CAR inhibited NAFLD/NASH-induced HCC in mice (Kettner et al., 2016; Takizawa et al., 2011).

8. Future directions of fatty liver disease treatment targeting nuclear receptors

Pharmacological activation of NR1 is expected to attenuate hepatic steatosis, inflammation, fibrosis, insulin resistance, dyslipidemia and obesity. However, some nuclear receptor agonists exhibited insufficient or paradoxical effects. For example, PPAR α activation is basically beneficial for fatty liver disease, but the effects of fibrates for human NAFLD are limited. PPAR γ activation improves adipocyte function, but enhances hepatocyte steatosis. Such unexpected findings may be derived from low potency and/or low specificity for the

target organ/tissue. In order to increase beneficial effects, the development of more potent and more stable single agonist, and possible combination therapies, such as PPAR α agonist + PPAR γ agonist, FXR agonist + PPAR γ agonist, and PPAR pan-agonist + LXR antagonist, should be examined. For the development of drugs to stimulate/inhibit nuclear receptors more effectively, improvement of organ/tissue specificity should be considered. Furthermore, strategies targeting epigenetic and post-transcriptional modulations and stimulation of nuclear receptor counterparts, such as co-activators and RXR α , might provide promising therapeutics in the future.

9. Conclusion

We reviewed the role of NR1 for the development of fatty liver disease. Nuclear receptor dysregulation contributes to the pathogenesis of NAFLD/NASH by impacting the integrated control of energy/nutrient metabolism thorough the gut-liver-adipose axis and inflammatory signaling. Nuclear receptor-targeted therapies may be beneficial for fatty liver disease, but the effectiveness is still unsatisfactory. Novel pharmacological interventions, such as dual/triple agonists, combination of agonists/antagonists, tissue-specific agonists/antagonists, and nuclear receptor modulators, would be critical to obtain benefits of nuclear receptor activation while minimizing adverse metabolic effects.

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Abbreviations:

ABC	ATP-binding cassette transporter
ACACA	acetyl-CoA carboxylase α
ACACB	acetyl-CoA carboxylase β , ACLY, ATP citrate lyase
ACADM	medium-chain acyl-CoA dehydrogenase
ACOX1	acyl-CoA oxidase 1
AFB1	aflatoxin B1
AMPK	adenosine monophosphate-activated protein kinase
ALD	alcoholic liver disease
ALT	alanine aminotransferase
ASH	alcoholic steatohepatitis

αSMA	α -smooth muscle actin
BA	bile acid
CAR	constitutive androstane receptor
CCl₄	carbon tetrachloride
CDCA	chenodeoxycholic acid
CPT1A	carnitine palmitoyl-CoA transferase 1 α
CTLN2	adult-onset type II citrullinemia
CYP	cytochrome P450
CYP7A1	cholesterol 7 α hydroxylase
DR	direct repeat
DSS	dextran sulfate sodium
FA	fatty acid
FABP	fatty acid-binding protein
FASN	fatty acid synthase
FGF	fibroblast growth factor
Fra	Fos-related antigen
FSP27	fat-specific protein 27
FXR	farnesoid X receptor
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDL	high-density lipoprotein
Hes6	hairy and enhancer of split 6
HSC	hepatic stellate cell
IBD	inflammatory bowel disease
IL	interleukin
LXR	liver X receptor
MCD	methionine and choline-deficient
MCP1	monocyte chemoattractant protein 1

NAFL	non-alcoholic fatty liver
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NF-κB	nuclear factor kappa B
NRF2	nuclear factor erythroid 2
OCA	obeticholic acid
OST	organic solute transporter
PGC1α	PPAR γ coactivator 1 α
PD	pancreaticoduodenectomy
PP	peroxisome proliferator
PPAR	peroxisome proliferator-activated receptor
PXR	pregnane X receptor
RAR	retinoic acid receptor
RXR	retinoid X receptor
SHP	small heterodimer partner
SLC25A20	carnitine-acylcarnitine translocase
SREBP	sterol-regulatory element-binding protein
TAG	triacylglycerol
TLR	Toll-like receptor
TNFα	tumor necrosis factor α
TZD	thiazolidinedione
WAT	white adipose tissue

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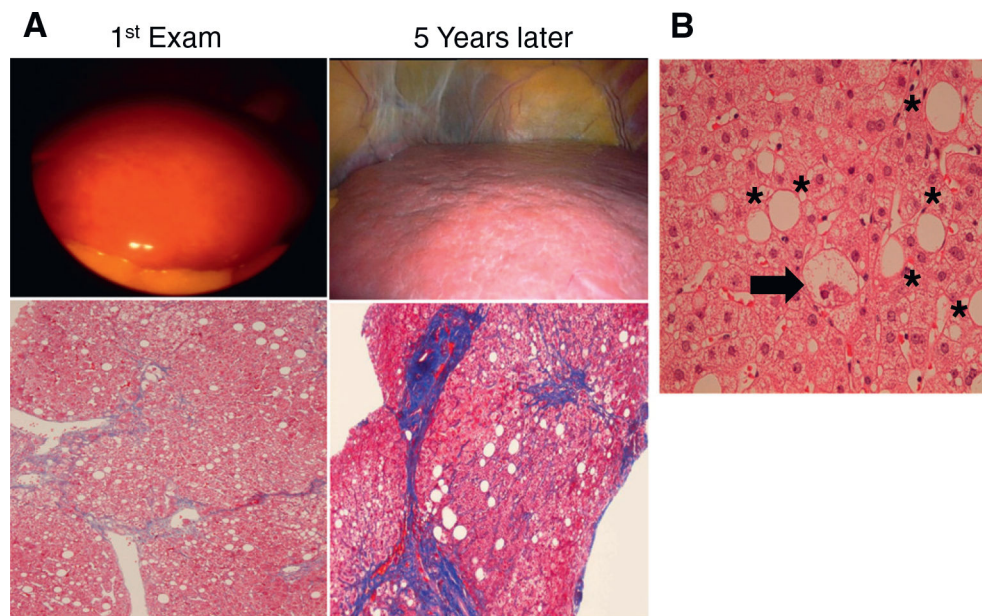


Fig. 1. Clinical course of NAFLD/NASH. A. The patient had obesity, diabetes, hypertension, dyslipidemia, and persistent elevation of serum alanine aminotransferase (ALT) levels. Initial laparoscopic examination revealed yellowish enlarged liver with smooth surface and soft consistency (1st Exam, upper panel), while liver biopsy section showed steatosis without significant fibrosis (1st Exam, lower panel). Serum ALT levels did not improve and the laparoscopic examination carried out at 5 years after the initial biopsy revealed whitish liver with rough surface (5 years later, upper panel). Dense fibrotic bands were found in biopsied specimen, indicative of pre-cirrhotic phase (5 years later, lower panel). The sections in the lower panel are stained by the Azan-Mallory method, and collagen fiber are indicated as blue. B. Careful pathological examination of the first biopsied specimen detected hepatocyte ballooning (arrow) in addition to macrovesicular steatosis (*), leading to the diagnosis of steatohepatitis. This section is stained by the hematoxylin and eosin method.

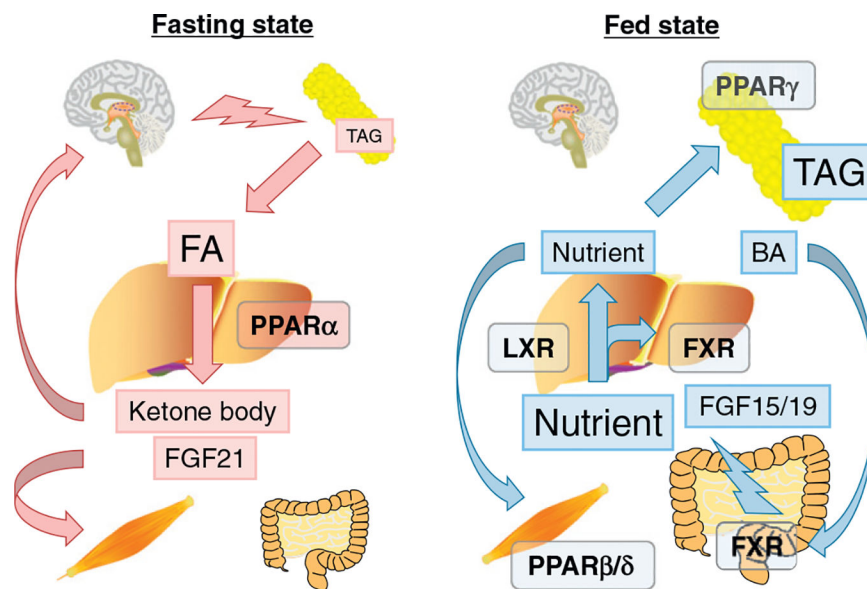


Fig. 2. Nuclear receptors as energy vectors. In a fasting state, triacylglycerol (TAG) stored in white adipose tissue is subjected to lipolysis and released into the circulation as fatty acids (FAs). FAs are taken into many organs as an energy source. In the liver, FAs activate PPAR α and enhance FA catabolism, resulting in the production of ATP, ketone bodies, and hepatokine fibroblast growth factor (FGF) 21. Ketone body is consumed as an energy source in the brain and FGF21 serves as a stress messenger to prepare other organs for energy deprivation. In the fed state, energy flux is reversed and FXR, LXR, PPAR β/δ and PPAR γ are mainly involved in nutrient absorption from the gut and distribution from gut/liver to peripheral tissues, such as adipose tissue and muscle. After meals, bile acids (BAs) activate intestinal FXR, promoting nutrient absorption and maintaining a barrier to the gut microbiome. Absorbed dietary lipids are transported into the circulation as chylomicron and its remnant. Hepatic FXR promotes post-prandial TAG-rich lipoprotein clearance. Excess cholesterol is removed from the body by reverse cholesterol transport under the control of the FXR-stimulated enterokine FGF19 (FGF15 in rodents) and/or activation of hepatic LXR by oxysterols. FGF15/19 attenuates post-prandial hyperglycemia through enhancing hepatic glycogenesis. Consequently, excess nutrients are either consumed in muscle or stored in WAT due to PPAR β/δ and PPAR γ , respectively. The concept “dysfunction of energy vectors on the gut-liver-adipose axis” may explain the pathogenesis of NAFLD/NASH.

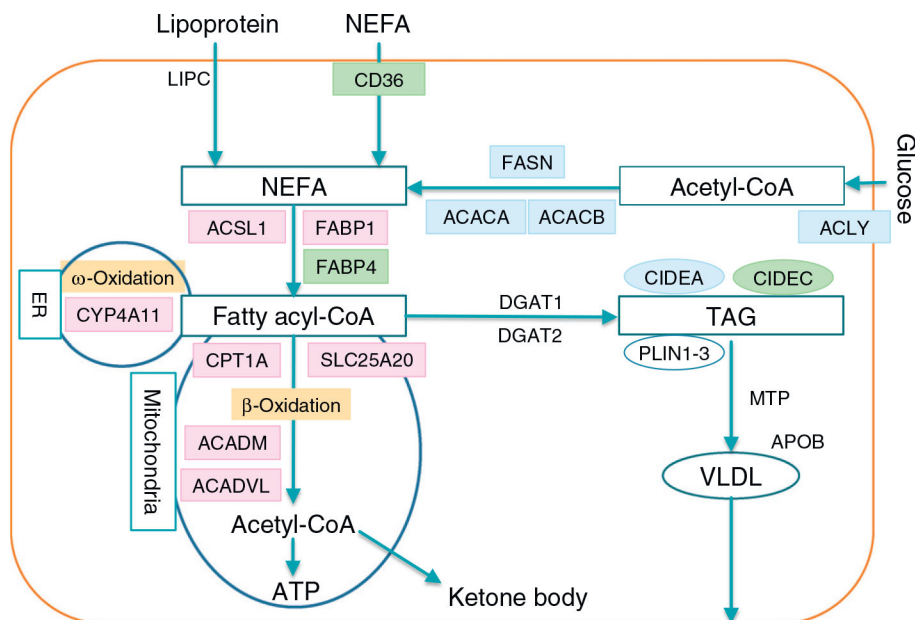


Fig. 3.

FA/TAG metabolism in hepatocytes. Non-esterified fatty acids (NEFAs) are taken from blood into hepatocytes or synthesized from glucose. NEFAs are converted to fatty acyl-CoA esters, then are subjected to β -oxidation (Only mitochondrial β -oxidation pathway is shown for simplification), or generation of triacylglycerol (TAG) and very-low-density lipoprotein (VLDL). TAG is stored in the hepatocytes in the form of lipid droplets, which are coated by perilipin 1–3 and cell death inducing DFFA like effector (CIDE) a-c to prevent excessive lipolysis. The genes regulated by PPAR α , PPAR γ , and SREBP1c are indicated in pink, green, and blue, respectively. PPAR α activation drives FA elimination, but the signaling of PPAR γ and SREBP1c stimulates lipogenesis and TAG storage. ACACA, acetyl-CoA carboxylase α ; ACACB, acetyl-CoA carboxylase β ; ACADM, medium-chain acyl-CoA dehydrogenase; ACADVL, very-long-chain acyl-CoA dehydrogenase; ACLY, ATP citrate lyase; ACSL1, long-chain acyl-CoA synthetase; APOB, apolipoprotein B; CIDEA, cell death inducing DFFA like effector a; CIDEC, cell death inducing DFFA like effector c; CPT1A, carnitine palmitoyl-CoA transferase 1 α ; CYP, cytochrome P450; DGAT, diacylglycerol acyltransferase; FABP, fatty acid-binding protein; FASN, fatty acid synthase; LIPC, hepatic lipase; MTP, microsomal triacylglycerol transfer protein; NEFA, non-esterified fatty acid; PLIN, perilipin; SLC25A20, carnitineacylcarnitine translocase; TAG, triacylglycerol; VLDL, very-low-density lipoprotein.

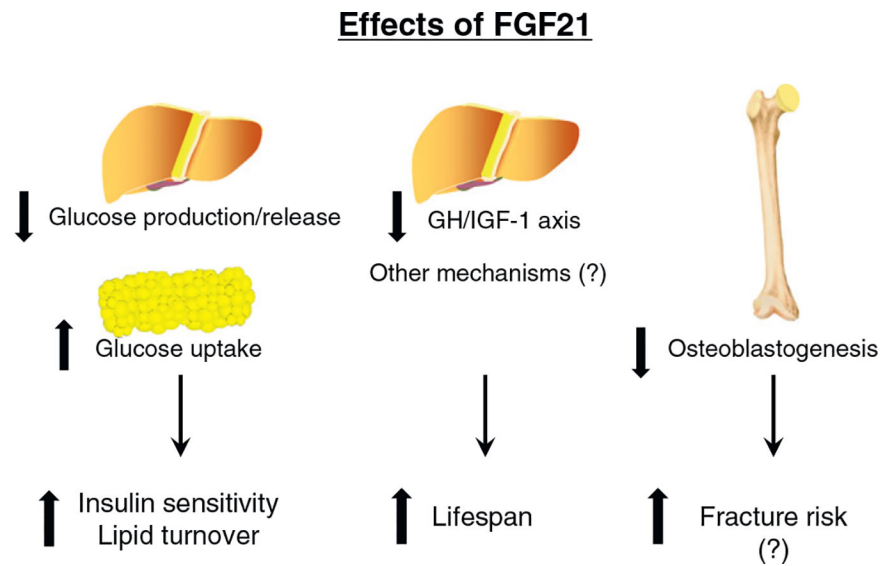


Fig. 4. Effects of FGF21. A hepatokine FGF21 is secreted into blood in response to various stress, such as fasting and endoplasmic reticulum stress, and activation of PPAR α . It binds to a plasma membrane receptor complex, mainly FGF receptor 1 and β -Klotho, and enhances expression of glucose transporter 1 in extra-hepatic tissues, leading to improvement of systemic insulin sensitivity and enhancement of lipid turnover. FGF21 may prolong lifespan through down-regulating growth hormone (GH)-insulin-like growth factor 1 (IGF-1) axis or other unknown mechanisms. FGF21 may inhibit osteoblastogenesis and promote osteoclastogenesis, causing increased fracture risk, while it remains controversial.

Table 1

Classification of nuclear receptors.

Nomenclature	Abbreviation	Name
NR0		
NR0B1	DAX-1	Dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1
NR0B2	SHP	Short heterodimeric partner
NR1		
NR1A1	TR α	Thyroid hormone receptor α
NR1A2	TR β	Thyroid hormone receptor β
NR1B1	RAR α	Retinoic acid receptor α
NR1B2	RAR β	Retinoic acid receptor β
NR1B3	RAR γ	Retinoic acid receptor γ
NR1C1	PPAR α	Peroxisome proliferator-activated receptor α
NR1C2	PPAR β/δ	Peroxisome proliferator-activated receptor β/δ
NR1C3	PPAR γ	Peroxisome proliferator-activated receptor γ
NR1D1	REV-ERB α	Reverse-Erb α
NR1D2	REV-ERB β	Reverse-Erb β
NR1F1	ROR α	RAR-related orphan receptor α
NR1F2	ROR β	RAR-related orphan receptor β
NR1F3	ROR γ	RAR-related orphan receptor γ
NR1H2	LXR β	Liver X receptor β
NR1H3	LXR α	Liver X receptor α
NR1H4	FXR α	Farnesoid X receptor α
NR1H5	FXR β	Farnesoid X receptor β (pseudogene)
NR1I1	VDR	Vitamin D receptor
NR1I2	PXR	Pregnane X receptor
NR1I3	CAR	Constitutive androstane receptor
NR2		
NR2A1	HNF4 α	Hepatocyte nuclear factor 4 α
NR2A2	HNF4 γ	Hepatocyte nuclear factor 4 γ
NR2B1	RXR α	Retinoid X receptor α
NR2B2	RXR β	Retinoid X receptor β
NR2B3	RXR γ	Retinoid X receptor γ
NR2C1	TR2	Testicular orphan receptor 2
NR2C2	TR4	Testicular orphan receptor 4
NR2E1	TLX	Tailless homolog orphan receptor
NR2F1	COUB-TF α	Chicken ovalbumin upstream promoter-transcription factor α
NR2F2	COUB-TF β	Chicken ovalbumin upstream promoter-transcription factor β
NR2F6	COUB-TF γ	Chicken ovalbumin upstream promoter-transcription factor γ
NR3		
NR3A1	ER α	Estrogen receptor α
NR3A2	ER β	Estrogen receptor β

Nomenclature	Abbreviation	Name
NR3B1	ERR α	Estrogen related receptor α
NR3B2	ERR β	Estrogen related receptor β
NR3B3	ERR γ	Estrogen related receptor γ
NR3C1	GR	Glucocorticoid receptor
NR3C2	MR	Mineralocorticoid receptor
NR3C3	PR	Progesterone receptor
NR3C4	AR	Androgen receptor
NR4		
NR4A1	NGF1- β	Nerve-growth-factor-induced gene B
NR4A2	NURR1	Nur-related factor 1
NR4A3	NOR-1	Neuron-derived orphan receptor 1
NR5		
NR5A1	SF-1	Steroidogenic factor 1
NR5A2	LRH-1	Liver receptor homolog-1
NR6		
NR6A1	GCNF	Germ cell nuclear factor

Table 2

PPARs and NAFLD/NASH.

	PPARα	PPARβ/δ	PPARγ
Main tissue	Liver Heart	Muscle Skin	Adipose Macrophage
Natural ligands	FA Eicosanoids	FA Eicosanoids	Prostaglandin Eicosanoids
Synthetic ligands	Wy-14643 Fibrates	GW501516 GW0742, KD3010	Thiazolidinedione GW1929, GW2090
Hepatic steatosis	↓	↓	↑
Insulin resistance	↓	↓	↓
Obesity	↓	↓	↑
Cholesterol metabolism	↓	↓	~
Hepatic inflammation	↓	↓	↓
Hepatic fibrosis	↓ [?]	↓ [?]	↓
Hepatic cancer	↓ [?] ↑ (rodents)	↓ [?]	↓
Drugs for human NASH	Elafibranor (dual PPAR α/δ agonist)		Pioglitazone

“?” means controversial or limited data.