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The role of the retinoid receptor, RAR/RXR heterodimer, in liver physiology

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Abstract

Activated by retinoids, metabolites of vitamin A, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs) play important roles in a wide variety of biological processes, including embryo development, homeostasis, cell proliferation, differentiation and death. In this review, we summarized the functional roles of nuclear receptor RAR/RXR heterodimers in liver physiology. Specifically, RAR/RXR modulate the synthesis and metabolism of lipids and bile acids in hepatocytes, regulate cholesterol transport in macrophages, and repress fibrogenesis in hepatic stellate cells. We have also listed the specific genes that carry these functions and how RAR/RXR regulate their expression in liver cells, providing a mechanistic view of their roles in liver physiology. Meanwhile, we pointed out many questions regarding the detailed signaling of RAR/RXR in regulating the expression of liver genes, and hope future studies will address these issues.

Keywords

Retinoic acid receptors; Retinoid X receptors; Gene regulation; Liver physiology; Lipid metabolism; Bile acid hemostasis; Liver fibrogenesis; Nuclear receptors

1. Introduction

Retinoic acids (RAs), the major active metabolites of vitamin A, play a pivotal role in many essential biological processes, including embryogenesis, organogenesis, cell growth, differentiation and apoptosis. The biologic effects of RAs are mainly mediated through nuclear receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). Both RARs and RXRs have three isotypes, −α, −β, and −γ, that are encoded by different genes in humans and rodents, i.e. NR1B1–3 and NR2B1–3, respectively. In addition, each isotype also has multiple variants due to alternative promoter and splicing. Despite these differences, RAR isotypes share over 90% identity in their DNA binding domain

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Declaration of competing interest

The authors accept the invitation to submit an invited article for BBA on nuclear receptors RAR/RXR.

(DBD), followed by ~85% identity in the ligand binding domain (LBD) whereas the N- and C- terminal regions (also call A and F domains) vary markedly. Fig. 1 illustrates the modular structure of these domains, designated A-F. Similarly, RXR isotypes also share high sequence identity in their DBD and LBD domains. In contrast, the sequence identity in DBD and LBD between RAR and RXR are 60% and 27%, respectively despite both being activated by RAs and their metabolites [1].

All-trans retinoic acid (atRA) is believed to be the endogenous ligand for RARs as it activates RARs at nanomolar levels, a physiologically relevant concentration, although the EC50 for each isotype differs slightly [2]. Other stereoisomers of RA also activate RARs, including 9-cis RA and 13-cis RA [3]. Fig. 1B demonstrates that the residues that directly interact with atRA in RAR are well conserved among the isotypes [4]. However, the endogenous ligand for RXRs remains debatable. Although 9-cis RA was initially identified as a ligand for RXRs by in vitro assays [5], its concentration in plasma and tissues is either undetectable or below the levels used in in vitro assays [6]; nevertheless, atRA can isomerize to 9-cis RA in vivo. In contrast, polyunsaturated fatty acids (PUFA) have been shown binding also activating RXRα both in tissue extracts and gene reporter assay [7-9], including docosahexaenoic acid and arachidonic acid. These observations indicate that PUFA are endogenous ligands for RXRs. This speculation is further supported by a recent report where deficiency of vitamin A has limited effects in RXRα activation in vivo. In contrast, deficiency of PUFA greatly repressed RXRα activation [10].

As type II nuclear receptors, RARs are obligated to form heterodimers with RXRs and bind to retinoic acid response elements (RAREs), a sequence in the promoter of the target genes that regulates their expression, whereas RXRs can form homodimers and regulate gene expression. In vitro assays indicate that a given RAR isotype is able to heterodimerize with any of the RXR isotypes and bind to a RARE [11]. The consensus RARE favors a direct repeat (DR) of AGGTCA with a gap of 1–5 nucleotides (DR1–5) (Fig. 1). At resting state, RAR/RXR may function as a repressor of gene transcription because of the associated corepressors. Upon ligand binding, its LBD undergoes a conformation change, resulting in the release of corepressors and recruitment of coactivators, leading to activation of gene transcription. Because RXR is the obligated partner for RAR, agonists of RXR can permissively activate RAR and regulate its target's expression. This is also true for a few other members of class II nuclear receptors, including PPARs, LXRs, and FXRs [12-15]. As these receptors are the subject of other reviews, we will not discuss RA's role in the activation of these receptors here.

Both RAR and RXR are broadly expressed in almost all tissues, but the relative abundance of each isotype differs. Table 1 lists the expression pattern of these isotypes and the phenotype of the gene knockout in mouse. Numerous studies in the past few decades, summarized in many excellent reviews, have demonstrated the functional significance of RA mediated RAR/RXR activation in a wide variety of biological processes, including embryo development, homeostasis, cell proliferation, differentiation and death. In this review, we focus on RAR/RXR's role in liver physiology where all of their isotypes are expressed (Table 1).

2. RAR/RXR in the regulation of lipid metabolism

The liver is the major organ involved in lipid metabolism. Both RARα and RXRα, which are abundantly expressed, play an important role in regulating the expression of genes involved in hepatic lipid metabolism and homeostasis. Although deficiency of specific RAR isotypes in mice does not show any liver-specific phenotype, likely due to functional compensation from other RAR isotypes [21], mice with liver-specific knockout of RXRα, the most abundantly expressed isoform in the liver, exhibit increased hepatic levels of triglyceride and cholesterol [34]. Table 2 lists RAR/RXR target genes involved in lipid metabolism, which are also depicted in Fig. 2.

2.1. Regulation of fatty acids and triglycerides by RAR/RXR

Several independent studies have indicated that the retinoid signaling modulates energy expenditure and lipid metabolism in rodents as the administration of RA ameliorates obesity and glucose intolerance and suppresses adipose lipid stores in mouse models of obesity and diabetes [35-37]. Impaired hepatic retinoid signaling is also linked with non-alcoholic fatty liver disease in humans [38], further supporting the functional significance of RA in normal liver physiology. Subsequent studies indicate that this signaling is mediated through fibroblast growth factor 21 (FGF21), a hormone secreted by the liver, involved in gluco-neogenesis, lipid metabolism, and ketogenesis. As a hormone, FGF21 regulates gene expression through activation of its membrane receptors, FGF receptor (FGFR) 1 and β-Klotho. The target genes in FGF21/FGFR1-β-Klotho signaling pathway include glucose-6-phosphatase, phospho-enolpyruvate carboxykinase, carnitine palmitoyl transferase 1α, and 3-hydroxybutyrate dehydrogenase type 1. Pharmacological administration of FGF21 improves insulin sensitivity, normalizes plasma lipids levels, causes weight loss, and increases whole-body energy expenditure in obese rodents or monkeys [39-43].

Hepatic expression of FGF21 is directly regulated by RAR/RXR in both human and mouse. Li et al. reported that both RARα and β but not γ stimulated FGF21 expression in HepG2 cells [44]. A DR1 RARE is identified in human FGF21 promoter region at −644 to −632 nt upstream of the translation initiation site in gene reporter assays. Mutation of this RARE abolishes RA induction of this promoter. The functional role of RAR in FGF21 expression regulation is further confirmed in vivo in mouse liver, where over-expression of RARβ with a viral vector significantly elevated hepatic levels of Fgf21, leading to increased energy expenditure by promoting hepatic fatty acid oxidation and ketogenesis. Two putative DR5 RAREs are identified in the mouse Fgf21 promoter at −602 to −586 and −537 to −521 nt, by Chromatin immunoprecipitation (ChIP)-PCR analysis [44].

Like FGF21, FGF19 (Fgf15 in rodents) is another growth factor that plays an important role in modulating hepatic lipid, bile acid, and carbohydrate metabolism. Human hepatocytes and cholangiocytes express FGF19 [45], and RA stimulates FGF19 mRNA expression in both human hepatocytes and HepG2 cells [45]. A DR5 RARE (+3423 to +3439 bp in intron 2) is identified in the human FGF19 gene. Mutation of this RARE significantly reduced 9-cis RA-induction of its promoter activity in a gene reporter assay [46]. However, this RARE is not conserved in its mouse ortholog, suggesting that RARs do not directly regulate Fgf15 expression in this species.

The liver is one of the major organs that synthesizes Apolipoprotein C-III (Apo C-III), which facilitates hepatic very-low-density lipoprotein (VLDL) particle formation and secretion, thus affecting the levels of hepatic and plasma triglycerides. Vu-Dac et al. [47] demonstrated that retinoids increase human Apo C-III mRNA expression by activating RXR, thereby contributing to the hypertriglyceridemic effect of retinoids. Takahashi et al. [95] further identified a DR1 RARE in the human Apo C-III promoter. Interestingly, this DR1 RARE only binds RXR/RXR homodimers. Mutation of this DR1 RARE abolishes 9-cis RA stimulation of RXR-mediated Apo C-III transcription. In contrast, AM580, a RARα specific agonist, repressed the expression of Apo C-III and HNF4α in HepG2 and Hep3B cells, in association with increased expression of the small heterodimer partner (SHP/NR0B2) [48]. Decreased hepatic expression of Apo C-III and HNF4α was also found in vivo in mice fed a high-fat diet when administrated with AM580, where lower plasma levels of triglycerides (TG) and cholesterol were also seen. Because HNF4α is a positive transcription factor for hepatic Apo C-III expression, and because SHP blocks HNF4α transcriptional activity, the authors speculated that AM580 reduces Apo C-III expression by activating RARα, leading to up-regulation of SHP that then results in repressing HNF4α. However, it remains unclear how RARα stimulates SHP expression, an important player in modulating the expression of genes involved in maintaining lipid and bile acid homeostasis.

In addition, RAR also plays a role in de novo lipogenesis (DNL), the first step in lipid metabolism, mediated through the hairy and enhancer of split 6 (Hes6), a transcriptional repressor of HNF4α. This leads to reduced expression and activity of peroxisome proliferator-activated receptor-γ (PPARγ), a potent lipogenic transcription factor [49]. Three putative RAREs were identified in the proximal promoter of mouse *Hes6* gene using a gene reporter assay and ChIP-PCR. Most importantly, over-expression of RARα or treatment with atRA in vivo significantly reduced hepatic fat accumulation in obese mouse models, demonstrating the physiological significance of this regulation. However, it is not known whether this HES6-HNF4α-PPARγ-mediated pathway is conserved in humans.

2.2. Regulation of cholesterol metabolism

The liver is also the principal site for cholesterol metabolism. Both the ATP-binding cassette (ABC) transporter A1 (ABCA1) and G1 (ABCG1) mediate the efflux of cellular cholesterol to apolipoproteins (Apo) A-I and A-II, two plasma proteins that are the major components of high-density lipoprotein (HDL). These four proteins are predominantly synthesized in the liver, where their expression is directly regulated by RAR and/or RXR. Rottman et al. [50] first reported that retinoids stimulate Apo A-I expression in HepG2 cells. At least three RAREs have been identified in the apoA-I promoter. One of the RARE (located from −214 to −192 bp upstream of transcription start) is mostly activated by RXR alone, whereas the other two (located from −169 to −146 bp, and −134 to −119 bp) are responsible for the stimulatory effects of RARα and β [50]. In the case of apo A-II, only one RARE is identified in its promoter region and it only binds RXR [51]. In addition, retinoids also induce the expression of the cholesterol efflux transporters ABCA1 and ABCG1 in macrophages. Costet et al. found that atRA and TTNPB, a synthetic RAR agonist, increased ABCA1 mRNA and protein expression in both human and mouse macrophages. This up-regulation is mediated through a DR4 RARE in the human ABCA1 promoter, a site that

LXR/RXR also binds to. ChIP analysis in macrophages revealed that RARγ/RXR bind to this DR4 element in the presence of atRA, where weaker binding of RARα/RXR was also found. In contrast, RARβ/RXR did not show any binding to this site [52]. The RAREs in the human ABCG1 promoter were characterized by Ayaori et al. There are two RAREs localized in the ABCG1 gene upstream of exon 1 in promoter A and upstream of exon 5 in promoter B. While promoter A only responds to atRA with minor transcription activity, promoter B can be activated by both atRA and other RAR agonists, TTNPB, and AM580, which generates the major transcript. Gene reporter assays have confirmed that ABCG1 level is regulated more by promoter B than promoter A, where the identified a DR4 RARE in the promoter B overlaps with a liver X receptor-responsive element (LXRE). This RARE was further confirmed by ChIP assay and overexpression of RAR isoforms in reporter assays [53].

Sterol 27-hydroxylase (CYP27A1) catalyzes the degradation of cholesterol, an important pathway in sterol elimination. It is also the initiating enzyme in the acidic pathway that converts cholesterol to bile acids. CYP27A1 is highly expressed in human macrophage. Several studies have found that retinoids and synthetic RAR agonists (e.g. AM580, TTNPB) induce CYP27A1 expression and increase 27-hydroxy-cholesterol production in human macrophages, including primary cells and THP-1 cell lines [54-56]. At least one RARE, which is also shared with PPAR/RXR is identified in the proximal promoter of this human gene, localized between −853 and −217 bp upstream of its transcription start site. Both ChIP assays and reporter assays indicate that a DR1 RARE plays the dominant role in response to retinoid stimulation of RAR/RXR [54]. A recent study indicates that the activity of carboxylesterase 1 also modulates CYP27A1 expression in macrophages via the nuclear receptors PPARγ, RAR, and/or RXR [57].

3. RAR/RXR regulation of bile acid homeostasis

Bile formation is one of the most important functions of the liver. RAR/RXR regulates the expression of a number of genes involved in bile formation, including bile acid synthesis and transport (Table 3) (Fig. 2).

3.1. Regulation of bile acid synthesis

The classic pathway for bile acid biosynthesis occurs primarily in the liver, which contributes around 90% of bile acid production. Cholesterol 7a-hydroxylase (CYP7A1) is the rate-limiting enzyme in bile acid synthesis. The expression of CYP7A1 is transcriptionally regulated and tightly controlled. While HNF4α and LRH-1 activate CYP7A1 gene transcription, there are two separate signaling pathways that repress this transcription. The major one is mediated through FGF19 (Fgf15 in mouse) /FGFR4/β-Klotho signaling. In humans, both hepatocytes and gallbladder epithelia express FGF19, in addition to the ileal enterocytes. Although details of this regulatory mechanism remain unclear, when FGF19 activates its receptor FGFR4/β-Klotho on the plasma membrane of hepatocytes, this initiates a signaling cascade that transactivates MAPKs, resulting in repression of CYP7A1 expression [58]. A second pathway is mediated through the nuclear receptor SHP (NR0B2). SHP is not a usual nuclear receptor as it does not have a DNA

binding domain. However, when SHP interacts with other transcription factors, it blocks gene transcription, including HNF4α and LRH-1, two transcription factors that normally activate CYP7A1 expression. We have found that RA represses CYP7A1 expression in human hepatocytes and HepG2 cells by stimulating the expression of both FGF19 and SHP [45]. This repression is mediated through both FXR-dependent and -independent pathways. While Jahn's study [46] identified a novel DR5 RARE in human FGF19 gene as described earlier, it remains to be determined how a RAR agonist that stimulated SHP expression also can repress CYP7A1 expression.

The alternative acidic pathway of bile acid synthesis, accounting for around 10% of bile acid production, is initiated by CYP27A1, described in the previous section. In contrast to macrophages, we found that both bile acids and retinoids repressed CYP27A1 expression in human hepatocytes [45]. Chen and Chiang identified the bile acid response element in human CYP27A1 gene, localized at −147 bp of its promoter, a site that also binds HNF4α [59]. Because both bile acids and retinoids induce SHP expression in hepatocytes, it is likely that SHP represses HNF4α activity, leading to reduced CYP27A1 expression in hepatocytes. The different effects of retinoids on CYP27A1 expression in human hepatocytes and macrophages are likely due to cell-specific transcriptional regulatory mechanisms.

Similar to CYP7A1 and CYP27A1, retinoids also have inhibitory effects on several other genes involved in bile acid synthesis, including BAAT, CYP8B1, and AKR1D1 [60,61], where RAR or RXR may also play a role. This repressive regulation is likely mediated through RA-stimulated expression of SHP in hepatic parenchymal cells, although details remain elusive.

3.2. Regulation of hepatic bile acid transporters

The sodium taurocholate co-transporting polypeptide (NTCP, SLC10A1) and the multidrug resistance-associated protein 2 (MRP2, ABCC2), represent basolateral and apical membrane transporters respectively that are responsible for the uptake and efflux of bile acids, drugs and other organic anions from hepatocytes. Hepatic expression of NTCP and Mrp2 are induced by retinoids through activation of RAR/RXR heterodimer in vitro [62]. A DR5 RARE has been identified in the promoter of both rat Ntcp and Mrp2 genes [63]. RAR's role in NTCP expression is also seen in human cells. A DR5 RARE in human NTCP is localized to nucleotides −112 to −96 of its promoter. When a synthetic chemical Ro41-5253 antagonizes RAR transactivation, the expression of NTCP, which also functions as the receptor for human hepatitis B virus entry into the hepatocyte, is reduced in cultured human hepatic cells, leading to decreased infection of hepatitis B virus infection [64].

Moreover, in inflammatory conditions, NTCP is down regulated, mainly by IL-1β-induced suppression of RXR/RAR nuclear binding activity as shown in vitro. The retinoid response element of the NTCP promoter was identified at −158 to −36 nt upstream of the translation initiation site, which contains a putative DR2 RARE sequence [63]. Mutations of this DR2 RARE reduced the NTCP promoter activity and NTCP transcription. Besides direct induction by retinoids, transcription of NTCP is also suppressed by the FXR/RXR heterodimer via its up-regulation of SHP [65]. Recent studies have suggested that SHP can suppress RXR/RAR activity through a direct protein-protein interaction [66]. Thus, it

means that NTCP is also regulated by cross-talk between a retinoid-RAR-dependent direct pathway and a bile acid-FXR-mediated indirect pathway. However, the expression of NTCP is maximally regulated by bile acid-activation compared to retinoid-activation when both retinoids and bile acids are present [62].

Several lines of evidence indicate that decreased hepatic Mrp2 expression in cholestatic livers is also associated with the reduction of nuclear RARα/RXRα levels as seen with NTCP expression. The proinflammatory cytokine IL-1 β was identified as the responsible inducer of this down-regulation [67-69]. However, this regulatory mechanism is only seen in cholestatic livers, as down-regulation of NTCP and Mrp2 in CCl_4-treated rats is independent of nuclear RAR/RXR but regulated by HNF1 [69].

Organic anion transporting polypeptides (OATPs), OATP1B1 and 1B3, organic cation transporter 1 (OCT1) and organic anion transporter 2 (OAT2), are organic anion and cation transporters expressed on the basolateral membrane of the hepatocyte, which are also thought to be regulated in an RXR/RAR-dependent manner, as atRA treatment decreases mRNA expression of these transporters in both primary human hepatocytes and hepatoma HepaRG cells. Knockdown of RARα or RXRα in HepaRG cells using siRNA transfection diminishes atRA repression of the expression of these genes [70], providing corroborating evidence of involvement of these nuclear receptors. However, how atRA and RAR/RXR repress the expression of these genes has yet to be defined. We have also found that RAR/RXR represses human MRP3 (ABCC3) expression in gene reporter assays [71]. MRP3 is expressed on the basolateral membrane of hepatocytes. It effluxes intracellular organic anions and bile acid glucuronides. Its expression is increased in cholestatic livers as an adaptive response to protect the liver from toxic chemicals. Under normal physiologic conditions, RAR/RXR binds to Sp1, a transcription activator of MRP3. This protein-protein interaction blocks Sp1 transcription activity and represses MRP3 expression. However, in cholestasis, hepatic expression of RAR/RXR is reduced. This results in increased transcription activity of Sp1 with the MRP3 promoter, providing a mechanistic explanation for RAR/RXR's role in regulating MRP3 expression [71].

The apical sodium-dependent bile acid transporter (ASBT, SLC10A2), located on the luminal membrane of cholangiocytes as well as the terminal ileum, functions to actively reabsorb bile acids, thereby facilitating the cholehepatic and enterohepatic circulations of bile acids. Gene promoter reporter assays indicate that RAR/RXR positively regulates human ASBT mRNA expression. A DR2 RARE is identified from +118 to +131 nt downstream of the transcription initiation site in the human ASBT gene. RA stimulates this promoter activity by four-fold, while site-mutations of this DR2 RARE attenuate the basal activity of the promoter by 50% [62]. Further studies indicate that this DR2 RARE is also responsible for bile acid repression of ASBT expression as the mutation of this DR-2 RARE eliminated bile acid repression. This negative feedback regulation is mediated through the FXR signaling pathway. When FXR is activated, SHP and FGF19/Fgf15 expression is stimulated. Increased SHP in turn represses RAR/RXR activity in ASBT transcription, leading to this down-regulation [73-75]. In addition, elevated FGF19/Fgf15 represses ASBT expression in human cholangiocarcinoma Mz-ChA-1 cells and colon cancer Caco2 cells

through FGFR4/β-Klotho receptors as mentioned earlier, although details of this signaling pathway still remain unclear [76].

The bile salt export pump (BSEP, ABCB11) is the major apical transporter in the hepatocyte for the efflux of bile salts. FXR/RXR heterodimers activate transcription of human BSEP through binding to the IR-1 site [77]. Although FXR/RXR is thought to be the permissive RXR heterodimer, Kassam A et al. reported that RXR agonists didn't activate the FXR/RXR heterodimer, but prevented the binding of FXR/RXR heterodimers to the BSEP promoter, suggesting RXR-mediated antagonism for ligand-bound FXR-induced expression of BSEP in both rodent and human [78]. During the transactivation of the IR-1 element, the presence of ligand for the FXR/RXR complex requires the recruitment of steroid receptor coactivators, raising the possibility that RXR ligands may have an antagonistic effect on FXR activation [78].

In summary, RAR-, RXR- mediated pathways are involved in suppressing bile acid synthesis and stimulating bile acid export by directly or indirectly regulating enzymes (CYP7A1, CYP27A1, CYP8B1, BAAT) and transporters (NTCP, MRP2, MRP3, OATPs, and others), and thus can be interpreted as having anti-cholestatic effects.

4. RAR/RXR in the regulation of liver fibrogenesis

Lipid droplets in hepatic stellate cells (HSC) are the central site for the storage of retinoids in the body. Activated HSCs are characterized by loss of retinoids and production of extracellular matrix (ECM), and play an important pathogenic role in liver fibrosis. RXRs and RARs are expressed in quiescent HSCs from rodents and humans, where their alpha isoforms are most highly expressed. However, their expression is reduced when HSCs are activated [79].

When HSC are activated in vivo in the livers of patients with cirrhosis or carcinoma or in bile duct ligated cholestatic rats, the mRNA expression levels of RARβ and RXRα are down-regulated, suggesting that these nuclear receptors play a role in HSC activation and liver fibrogenesis [80,81]. Cortes et al. demonstrate that atRA promotes human HSC deactivation via RARβ-dependent transcriptional down-regulation of myosin light chain 2 (MLC-2) expression (Table 4). MLC-2 plays a major role in cytoskeletal tension, force generation, mechanosensing, and ECM deposition. Elevated hepatic expression of MLC-2 is associated with liver fibrosis in both humans and mice [81]. Consistent with these observations, reduced liver fibrosis is also seen in cholestatic rodent models after administration of atRA [82-84], where atRA demonstrates inhibitory effects on the expression of profibrotic genes, including TGF-β1, COL1A1, MMP2, and α-SMA both in vivo in rodent livers and in vitro in primary human hepatic stellate cells and LX-2 cells. Reduced mRNA expression of all three RAR isotypes is also found in freshly isolated rat HSC where treatment with retinoids decreased markers of activation [85,86]. In further support of RAR's role in HSC activation, Mezaki et al. found that RARα and β proteins formed insoluble aggregated speckled droplets in the cytosol of retinol activated rat HSC [87], suggesting that RARs have lost their function as transcription factors. Indeed, the

expression of RARα, RARβ and RARγ are undetectable in chronically activated rat HSC [86].

More direct evidence for RXR's role in HSC activation comes from over-expression of RXRα in transfected rat HSC cultures, which induces a quiescent phenotype [88]. RXR specific agonists are also able to inhibit cell proliferation in HSC. Similarly, treatment of activated rat HSC with atRA or RAR agonists both reduced the expression of profibrotic genes, including collagen I, collagen III, and fibronectin. AtRA also reduced HSC proliferation, whereas RAR agonists did not. In contrast, RAR specific antagonists enhance HSC proliferation, indicating that RARs do play a role in HSC proliferation [89]. Similarly, the combined treatment of ligands for PPAR, RAR, and RXR results in an anti-proliferative effect by inducing cell cycle arrest at the G0/G1 phase [90].

Two RAREs in mouse Collagen I alpha-2 chain (Col1a2) promoter have been identified. They are localized at −879 to −874 bp (site 1) and −930 to −911 bp (site 2). When the reporter construct was cotransfected with RARβ and RXRα expression vectors into stellate cells or the transfected cells were treated with RA, the promoter activity was suppressed. Conversely, mutation of these RAREs enhanced promoter activity, demonstrating the direct role of RAR/RXR in this gene expression regulation [91]. However, these two RAREs are not typical RAREs. Site 1 appears to be half of the regular RARE, whereas site 2 is an everted repeat of the conserved sequence AGGTCA with a gap of 8 base-pairs. Also, a liganded RAR/RXR normally transactivates gene expression rather than represses it. It is not known whether this repressive effect is due to these two atypical RAREs. Alternatively, the RAR/RXR repression of mouse Col1a2 is mediated through transrepression of another transcription factor, such as AP1 (Fig. 2). Several studies have indicated that AP1 positively regulates the expression of many genes involved in liver fibrosis, including TGF-β1, collagenase, stromelysin, and TNF-α [77,89-92], where retinoids repress the expression of these genes. This transrepression is thought to be mediated through RAR/RXR. In this case, liganded RAR does not directly bind to a traditional RARE in the promoter but rather associates with AP1 through protein-protein interactions [92-94]. When RAR binds to AP1, it blocks AP1 transcription activity, leading to reduced expression of the target genes. As a previously mentioned example, RAR/RXR counteracts Sp1 transactivation of MRP3 expression [71]. These regulatory mechanisms may also provide an explanation for atRA and RARβ repression of MLC-2 expression in HSC [81]. In summary, it is clear that RARs and RXRs play a major role in HSC activation by modulating cell proliferation and the expression of profibrotic genes, although many mechanistic details still remain to be determined.

5. RAR/RXR in the regulation of liver inflammation

Supplementation of RA has been shown to reduce liver inflammation in several animal models of liver disease, suggesting a potential role for RAR/RXR in regulating the immune response [101-103]. Many studies have indicated that RA plays a very important role in immune cells differentiation and activation [104,105]. In particular, RA represses the production of proinflammatory cytokines in immune cells, including dendritic cells, monocytes, macrophages, T-cells [3,105-108]. The anti-inflammatory effects of RA are

partially RAR/RXR dependent. Dawson et al. showed that repression of IFN-gamma and TNF-alpha by RAs is mediated through activation of RARα, but not RARβ or RARγ in human T cells because the RARα-selective agonist AM580, but not the RARβ/γ ligand 4-hydroxyphenylretinamide, recapitulates RA's effect [3]. Dzhagalov et al. revealed that RARγ is a positive regulator of inflammatory cytokine production in CD8+ T-cells and macrophages using RARγ-deficient mice [109]. Together, these observations indicate that individual retinoid receptors play specific roles in the differential regulation of immune responses. Several studies have also revealed that RXR alone or in association with RAR block NF-κB or AP-1 activation in immune cells [102,106-108,110], where both NF-κB and AP-1 are transactivators of proinflammatory cytokine expression. (Table 5).

The anti-inflammatory effects of RA are also found in microglia and astrocytes [111-113]. Reduced production of metalloproteases (MMP), cyclooxygenase-2 (COX-2), and prostaglandin I synthase (PGIS) were also found in other cells [110,114-116]. However, we were not able to find any reports in the literature regarding RAR/RXR's role in proinflammatory cytokine production in hepatic cells. Instead, several studies indicate that hepatic cells respond to proinflammatory cytokines by altering gene expression where RAR/RXR is involved as mentioned earlier in the regulation of NTCP, MRP2 and MRP3 expression. Furthermore, Aguirre and Karpen indicate that interleukin-1β and TNFα cause RXRα SUMOylation in hepatocytes, resulting in decreased expression of its target genes [117]. Clearly, more studies are needed to examine RAR/RXR's role in hepatic inflammation.

6. Conclusions and prospective

RAR and RXR are significant therapeutic targets for various clinical disorders, i.e., atopic dermatitis, breast cancer, acute promyelocytic leukemia (APL), and diabetic cardiomyopathy [118-121]. Emerging findings have shown that vitamin A homeostasis is implicated in the prevention of hepatic fibrosis, regulation of hepatic immunological response to cholestasis, and reduction of liver injury [83,84,122-124]. Given the crucial role of Vitamin A deficiency in chronic cholestasis, RAR and RXR may hold therapeutic potential for the treatment of cholestatic liver diseases. Although UDCA and Obeticholic acid have been approved by the FDA for early-stage and UDCA-refractory primary biliary cholangitis [125,126], not all patients respond, and beneficial effects on clinical endpoints in primary sclerosing cholangitis (PSC) patients remains controversial [127]. Thus there is a need for alternative therapies. Several lines of evidence indicate that atRA has potentially positive effects on liver injury in several rodent models of chronic cholestasis, including bile duct ligation and alpha-naphthyl isothiocyanate in the rat and in Mdr2 null mice, a model of PSC [84,128]. The most positive findings were seen in rats treated with atRA with and without UDCA, which demonstrated marked reductions in necrosis and hepatic fibrosis [83]. In addition, markers of inflammation and significant decreases in the bile acid pool and bile duct proliferation were also seen, with the combination of atRA and UDCA having the greatest effect. The regulatory mechanisms may involve suppressing the activity of cytochrome CYP7A1 [83,84], which led to reductions in the bile acid pool size and reduced bile duct proliferation. In addition, RA repressed collagen 1A1 expression in stellate cells suggesting an anti-fibrotic effect [83]. These encouraging findings led to a small clinical

trial that found that combination therapy of UDCA and atRA for 12 weeks in patients with PSC had an inhibitory effect on bile acid synthesis (C4) and significantly reduced serum aminotransferases, thus potentially reducing hepatic inflammation [129]. However, retinoids can also exhibit serious side effects such as teratogenicity, hypervitaminosis, and resistance can develop [130]. Therefore, several synthetic RAR- and RXR- ligands (Rexinoids) have been developed in recent years that show potential as anti-tumor agents in clinical and pre-clinical studies, including AM80 (dual RARα/β-selective agonist) for APL [131] and bexarotene (RXR ligands) for persistent cutaneous T-cell lymphoma [132]. It is possible that these specific ligands could also be promising new therapeutic agents in the therapy of chronic cholestatic disorders.

As summarized in this review, RAR and RXR play critical roles in many aspects of hepatic physiology and responsiveness to disease (Fig. 2). Here we have focused on those aspects that relate to hepatic lipid metabolism, bile acid metabolism and the pathogenesis of hepatic fibrosis and inflammation, as well as the possibility that ligands of these nuclear receptors may have potential therapeutic anti-inflammatory and anti-fibrotic benefits in liver disease. While many details of these interactions have been described, there is still much to be learned about these pathways and particularly where novel therapeutic interventions might be targeted. Given the widespread roles of RAR and RXR in the regulation of these hepatic functions, future studies should continue to focus on this important area.

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Abbreviations:

atRA	all-trans retinoic acid
ASBT/SLC10A2	apical sodium-dependent bile salt transporter
ABC	ATP-binding cassette transporter
Apo	apolipoprotein
AKR1D1	aldo-keto reductase family 1 member D1
AP-1	activator protein 1
BAAT	bile acid coenzyme A: amino acid N-acyltransferase
BSEP/ABCB11	bile salt export pump
CYP7A1	cholesterol 7-alpha-hydroxylase
CD36	cluster determinant 36
COL1A1	collagen type I alpha 1 chain
COL1A2	collagen type I alpha 2 chain

CE	cholesterol ester
DBD	DNA-binding domain
DR	direct repeat
DNL	de novo lipogenesis
ECM	extracellular matrix
FXR	farnesoid X receptor
FGF15/19	fibroblast growth factor 15/19
FGFR	fibroblast growth factor receptor
IR	inverted repeat
Hes6	hairy and enhancer of split 6
HDL	high-density lipoprotein
HNF4A	hepatocyte nuclear factor 4 alpha
HSC	hepatic stellate cells
IL	interleukin
LBD	ligand binding domain
LDLR	low-density lipoprotein receptor
LRH-1	liver receptor homolog-1
NTCP/SLC10A1	sodium/taurocholate cotransporting polypeptide
MRP	multidrug resistance-associated protein
MLC-2	myosin light chain 2
MMP-2	matrix metalloproteinase-2
OATP	organic anion transporting polypeptide
NF-κB	nuclear factor-kappa B
PPARγ	peroxisome proliferator-activated receptor gamma
PC	phosphatidylcholine
RA	retinoic acid
RARE	retinoic acid response element
RAR	retinoic acid receptor
RXR	retinoid X receptor

9cRA	9-cis retinoic acid
SHP/NR0B2	small heterodimer partner
CYP8B1	sterol 12-alpha-hydroxylase
CYP27A1	sterol 27-hydroxylase
α-SMA	alpha-smooth muscle actin
TNFα	tumor necrosis factor-alpha
Sp1	specificity protein 1
TGF-β1	transforming growth factor-beta 1
TG	triglycerides
VLDL	very-low-density lipoprotein.

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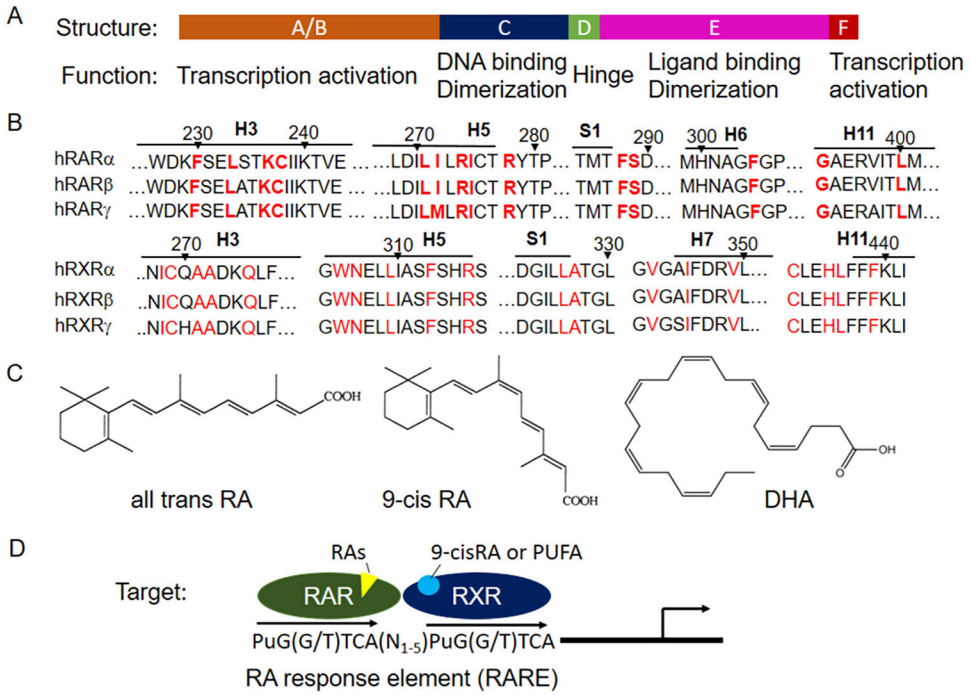

Fig. 1.

A structural model of retinoid receptors in gene regulation. A) structural modules of RAR and RXR. B) Residues that directly interact with atRA in RARs and 9-cis RA in RXRs are well conserved among isotypes within the family. C) Structure of atRA, 9-cis RA and docosahexaenoic acid (DHA, 22:6 omega-3). D) Transcription activation of target genes by RAR/RXR.

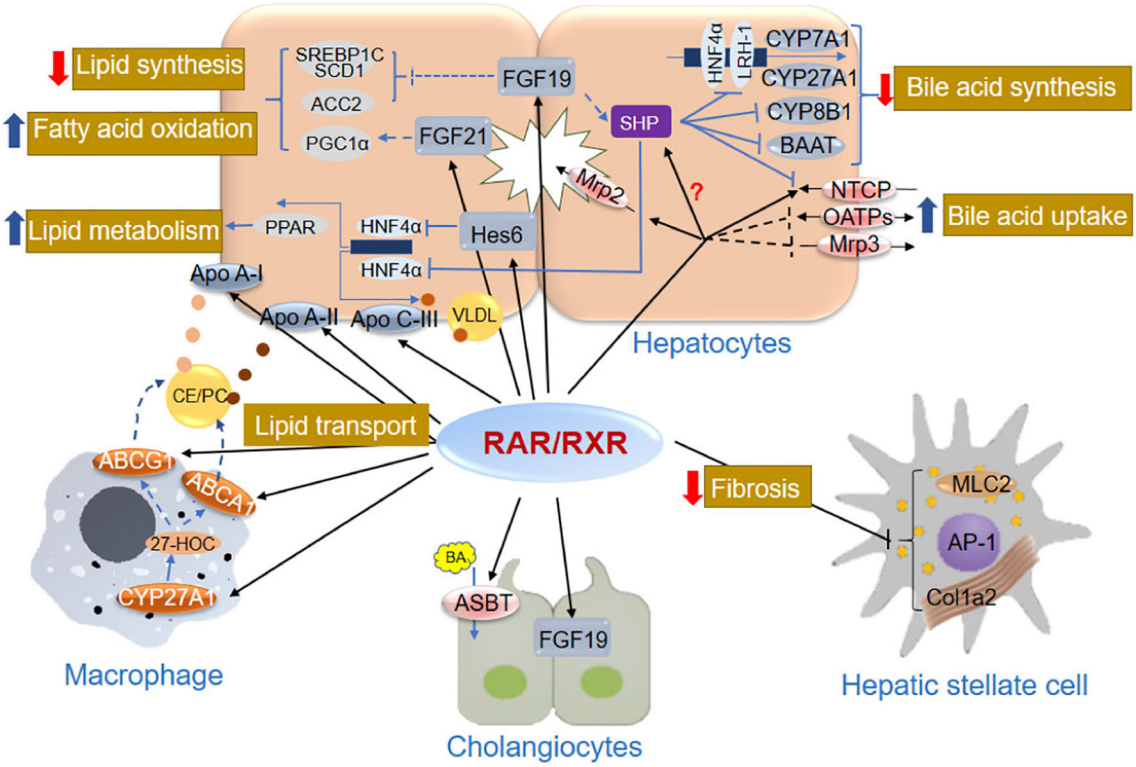

Fig. 2.

Functional role of RAR/RXR in liver physiology. The black arrows or bar-headed lines show key genes involved in lipid metabolism and fibrogenesis that are directly regulated by RAR/RXR signaling pathways in liver cells, including hepatocytes and cholangiocytes Kupffer cells, and stellate cells. Dashed lines indicate that genes are directly or indirectly regulated by RAR/RXR signaling pathways, the detail of which has not been defined. Thick red arrows mean inhibition, and thick blue arrows mean stimulation. Yellow, brown, and red dots represent apo A-I, apo A-II, and apo C-III, respectively. Abbreviations can be found separately in the “list of abbreviations.” More information about the regulations and function of the individual genes can be found in Tables 2-4.

Table 1

Tissue distributions of RARs and RXRs in mammals, and phenotypes of isotype-specific knockout in mouse.

Receptor (gene symbol)	Expression pattern		Knockout phenotype		Ref
	Most abundant	Liver	General	Liver specific	
RARα (NR1B1)	Widely expressed	Parenchyma, endothelia, kupffer and stellate cell	Postnatal lethality and some abnormalities including testis degeneration		[16-21]
RARβ (NR1B2)	Lung, intestine, genitalia, epithelia, limbs, brain.	Parenchyma, endothelial, Kupffer and stellate cell	No obvious external and internal abnormalities		[19,22-24]
PARγ (NR1B3)	Cartilage, embryo, epidermis, skeleton	Parenchyma, endothelial, Kupffer and stellate cell	Postpartum lethality and growth deficiency. (male sterility; Agenesis of the ocular Harderian gland and homeotic transformations of the axial skeleton)		[19,25-27]
RXRα (NR2B1)	Liver, kidney, spleen, placenta, epidermis and a variety of visceral tissues	Parenchyma, endothelial, kupffer and stellate cell	Died in utero and displayed myocardial and ocular malformations	Disturbed lipid metabolism; a deficiency of Epo expression in the fetal liver; impaired regenerative capacity	[19,28-30]
RXRβ (NR2B2)	Widely expressed	Parenchyma, endothelial, Kupffer and stellate cell	Normal. Except male infertility.		[19,30-32]
RXRγ (NR2B3)	Muscle, brain	Parenchyma, endothelial, and Kupffer cell	Postnatal lethality and some abnormalities including homeotic transformation		[19,27,32,33]

Note: The expression levels of all RXR subtypes is higher than all RAR subtypes in all liver cells.

Table 2

RAR/RXR target genes involved in hepatic lipid metabolism.

Target gene	Regulation	Function	Species	Cell type	References
FGF21 (RAR/RAR)	Stimulation	The hepatokine for promotion of fatty acid oxidation and suppression of lipid synthesis	Human	Hepatoma (hepG2)	[44]
			Mice	Hepatocytes	[44]
FGF19 (Fgf15 in rodent) (RAR/RXR)	Stimulation	The gut-derived hormone for suppression of lipogenesis and induction of fatty acid β-oxidation	Human	Ileum (HT-29 cell, caco-2 cell)	[45,46]
Apolipoprotein C-III (RAR/RXR, RXR/RXR)	Stimulation	The protein for inhibition of hepatic uptake of triglyceride-rich particles	Human	Hepatoma (Hep3B cells)	[48]
			Mice	Preadipocyte	[95]
Hes6 (RAR/RXR)	Stimulation	Transcriptional repressor for inhibition of HNF-4α transcriptional activity, subsequently repress HNF-4α-activated PPAR-γ2 expression	Mice	Hepatocytes	[49]
Apolipoprotein A-I (RAR/RXR)	Stimulation	The plasma protein for cholesterol and other lipids transportation in the plasma	Human	Hepatoma (HepG2)	[50]
ABCA1 (RAR/RXR)	Stimulation	The membrane protein for the efflux of cholesterol and phospholipids to lipidpoor apolipoproteins, generating nascent HDL particles.	Human	Macrophages (THP-1)	[53]
			Mice	Macrophages	[52]
ABCG1 (RAR/RXR)	Stimulation	The membrane protein for promoting cholesterol efflux from macrophages to HDL particles	Human	Macrophages (THP-1)	[53]
CYP27A1 (RAR/RXR)	Stimulation	The enzyme for degradation of cholesterol to bile acids	Human	Macrophages (THP-1)	[54-57]
CPT1 (RAR/RXR)	Stimulation	The rate-limiting enzyme in long chain fatty acyl-CoA uptake and oxidation in mitochondria	Mice	Hepatocytes	[96]

Table 3

RAR/RXR target genes involved in bile acid metabolism.

Target gene	Regulation	Function	Species	Cell type	References
CYP7A1\–/RAR/RXR)	Repression	The rate-limiting enzyme in the synthesis of bile acid from cholesterol by catalyzing the formation of 7α-hydroxycholesterol	Human	HepG2	[45,97]
				Hepatocytes	[45]
FGF19 (RXR/RAR)	Stimulation	The gut-derived hormone for inhibition of CYP7A1 expression via the FGFR4/Klotho-β receptor complexes in the liver	Human	Ileum (HT-29 cell, caco-2 cell)	[46]
CYP27A1 (RAR/RXR)	Repression	The enzyme for degradation of cholesterol to bile acids	Human	Hepatocyte	[45,59]
NTCP (RAR/RXR)	Repression	The major basolateral bile acid uptake transporter in hepatocytes	Human	HepG2	[62,63,69,98]
			Rat	Hepatocytes Stellate cell. Kupffer cell	[99,100]
			Mice	Hepatocytes	[65]
MRP2 (RAR/RXR)	Stimulation	The hepatic apical transporter for bilirubin, glutathione, bile acids and other organic anions and their conjugates	Human	HepG2	[63,69]
			Rat	Hepatocytes	[66]
MRP3 (RAR/RXR)	Repression	The hepatic basolateral pump for extrude bilirubin, bile acids and other organic anions and their conjugates	Human	HepG2	[71]
OATP1B1,1B3 (RAR/RXR)	Repression	The basolateral uptake transporter for organic anions	Human	Hepatocytes	[70]
OCT1 (RAR/RXR)	Repression	The basolateral uptake transporter for organic cations			
OAT2 (RAR/RXR)	Repression	The basolateral uptake of organic anions/dicarboxylate exchanger			
ASBT (RAR/RXR)	Stimulation	The apical bile acid uptake transporter in bile duct and ileum	Human	CT-26, Caco-2, HIBEC	[72-76]

Table 4

RAR/RXR target genes involved in liver fibrosis.

Target gene	Regulation	Function	Species	Cell type	References
AP-1 (RAR/RXR)	Repression	The transcription factor for regulating expression of collagenase, stromelysin, TGF-β1 and TNF-α. promoting fibrogenesis.	Rat	HSC	[80]
MLC-2 (RAR/RXR)	Repression	The sarcomeric protein for increasing HSC contractility (the RAR-β/MLC-2 axis)	Human	HSC	[81]
			Mice	HSC	
Col1a2 (RAR/ RXR)	Repression	A protein for the major component of extracellular matrix	Rat	HSC	[91]

Table 5

RAR/RXR target genes involved in liver Inflammation.

Target gene	Regulation	Function	Species	Cell type	References
IFN-γ (RAR/RXR)	Repression	The cytokine of the immune system related to the T helper type 1 (Th1) response to infection	Human	T lymphoblastoid cell	[106]
NF-κB (RAR/RXR)	Repression	The transcription factor for regulating expression of proinflammatory genes, including cytokines, chemokines, and adhesion molecules	Human	THP-1 cells	[108]
AP-1 (RAR/RXR)	Repression	The transcription factor for regulating expression of proinflammatory genes,	Human	THP-1 cells	[108]