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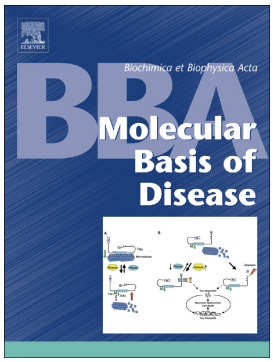

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Review

Nuclear Receptors in Liver Fibrosis

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Abstract:

Nuclear receptors are ligand-activated transcription factors that regulate gene expression of a variety of key molecular signals involved in liver fibrosis. The primary cellular driver of liver fibrogenesis are activated hepatic stellate cells. Different NRs regulate the hepatic expression of pro-inflammatory and pro-fibrogenic cytokines that promote the transformation of hepatic stellate cells into fibrogenic myofibroblasts. Importantly, nuclear receptors regulate gene expression circuits that promote hepatic fibrogenesis and/or allow liver fibrosis regression. In this review, we highlight the direct and indirect influence of nuclear receptors on liver fibrosis, with a focus on hepatic stellate cells, and discuss potential therapeutic effects of nuclear receptor modulation in regard to anti-fibrotic and anti-inflammatory effects. Further research on nuclear receptors-related signaling may lead to the clinical development of effective anti-fibrotic therapies for patients with liver disease.

Keywords:

Nuclear receptor, Liver fibrosis, FXR, VDR, PPAR, LXR, RXR, RAR, THR, GR, MR, AR, ER

1 Molecular and cellular signaling driving liver fibrosis

The key processes driving liver fibrosis include chronic inflammation and hepatic fibrogenesis, resulting in the accumulation of extracellular matrix (ECM; i.e. scar tissue) as well as functional and structural changes of parenchymal and non-parenchymal liver cells.[1] Chronic hepatocyte damage and cell death result in the release of damage-associated molecular patterns and apoptosis-related messenger molecules, which activate hepatic stellate cells (HSCs) and lead to the recruitment of immune cells.[2] Two of the prototype cytokines responsible for the activation of HSCs during liver injury are the transforming growth factor-β (TGF-β) and the platelet-derived growth factor (PDGF).[3] HSC activation results in an increased expression of contractile cytoskeleton filaments such as α-smooth muscle actin (αSMA) and ECM proteins including different types of collagen.[4] Once HSCs are activated

(aHSCs), they convert from quiescent vitamin-A storing mesenchymal cells into contractile myofibroblasts (MFs), characterized by enhanced migration, upregulated production of ECM components and the secretion of pro-inflammatory, pro-fibrotic and pro-mitogenic cytokines. Importantly, HSCs profoundly change their phenotype when transdifferentiating into MFs marked by an increased capacity for chemotaxis, fibrogenesis, contractility and a loss of cytoplasmatic retinoids.[5] While aHSCs represent the main source of fibrogenic MFs,[6] they may also derive from portal fibroblasts, bone marrow-derived cells, circulating fibrocytes,[7] and by the controversially discussed epithelial-to-mesenchymal (EMT)[8, 9] or endothelial-to-mesenchymal (EndoTM) transition.[10, 11]

Due to their central role in fibrosis, targeting the aHSC/MF has traditionally been regarded as a therapeutic strategy to prevent the progression of fibrosis. One approach may include the induction of apoptosis of aHSCs/MFs to ultimately inhibit their ECM deposition and stop the release of pro-fibrotic signal molecules. Alternatively, one may deactivate aHSCs and promote their return to a quiescent state (i.e., quiescent HSCs, qHSCs). Ultimately, the goal is prevention of pro-fibrotic influences to stop fibrogenesis or even to ensure liver fibrosis regression, which was observed in experimental[12, 13] as well as clinical studies[14, 15] in numerous diseases (e.g., after alcohol abstinence, in hepatitis C virus (HCV) patients after viral eradication in hepatitis C patients, or even just by a change in lifestyle)[16, 17]

Activation of HSCs is often mediated by immune cells as a response to liver injury through the secretion of pro-inflammatory & pro-fibrotic molecules, and by ECM components. Inflammatory cytokines are primarily secreted by immune cells such as Kupffer cells (KCs), natural killer cells or even hepatocytes (HCs) These cytokines include chemokines as monocyte chemoattractant protein-1 aka chemokine CC-motif ligand-2 (CCL-2) and CCL-5 or interleukins as IL-1, IL-6, IL-8, and IL-10 next to interferons (INF-α, INF-γ), several growth factors and adipokines.[2] Most inflammatory mediators either activate or target the

nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) which is a key transcriptional regulator of the inflammatory response.[18, 19]

The most potent activators of NF-κB resulting in HSC activation are pathogen-derived molecules (e.g., liposaccharide), inflammatory cytokines (e.g., tumor necrosis factor-α (TNF-α) and IL-1) or the DNA of virus/bacteria, all of which stimulate Toll-like receptors (TLRs), more specifically TLR-4 of qHSCs.[20] Thereby, NF-κB is a key molecule in the setting of chronic injury, inflammation and fibrosis, as well as in the apoptosis-survival regulation of HSCs and HCs. In aHSCs, NF-κB expression increases significantly and triggers the expression of pro-fibrotic and proinflammatory genes (IL-6, IL-8, mitochondrial substrate carrier protein 1 (MSCP-1), intercellular Adhesion Molecule 1 (ICAM1)). However, the decisive factor is that NF-κB-activation additionally causes a significantly increased resistance against apoptosis in aHSCs.[21] Whilst NF-κB gets autoregulated by the expression of inhibitor of nuclear factor kappa B (IκBα) in healthy tissues, this negative feedback loop is suppressed in aHSCs. This loss of autoregulation subsequently leads to a vicious circle in favor of fibrosis progression and aHSCs are unable to initiate cell death, therefore triggering continuous fibrogenesis.[22-24] Thus, NF-κB is another potential target in fibrosis treatment.

Modulation of anti-fibrotic pathways and cell-to-cell communication might be influenced by either deployment of transcription factors, such as nuclear receptors (NRs), histone deacetylation, DNA methylation or cell silencing by noncoding microRNAs. NRs in general, play a pivotal role in the overall control of several biological processes and represent a promising target in liver diseases.[25] Next to their extensive effect on cell metabolism, they are able to mediate anti-inflammatory effects through direct interaction with other transcription factors, such as NF-κB or activator protein-1 (AP-1)[26, 27], and even repress proinflammatory gene expression by interfering with TLR signaling.[28] Thus, NRs are a promising target

capable to intervene and modulate cell-based transcription in liver tissue resulting in the amelioration of liver diseases.

2 Nuclear receptors

NRs are the largest group of transcriptional regulators with 49 distinct, currently known subtypes NRs, that can be divided in 7 subfamilies by sequence homology [Figure-1A].[29, 30] In their function, NRs act as a sensor for small intracellular molecules and translate the respective signals to the genomic level.[31] The natural ligands that interact and regulate NR-activity are typically small lipophilic molecules, such as hormones, bile acids, oxysterols, fatty acids, vitamins, cholesterol, and exogenous substances, including toxins.[32] All NRs share a common structure which allows them to bind directly to DNA and regulate gene transcription.[25] This structure comprises [Figure-1B]: a N-terminal domain (NTD) with an activation factor-1 (AF-1) surface, a central DNA binding domain (DBD), a flexible hinge region and a C-terminal ligand binding domain (LBD) encompassed by an activation factor-2 (AF-2) surface.[33]

NRs can be sub-classified by their four major types of dimerization and binding sites on DNA, the so called transcription factor response element (TFRE) or hormone response element (HRE), which are localized in the regulatory region of target genes and typically contain two consens hexameric half-sites separated by 1-5 base pairs [Figure-1C].[34-36] Type-1 NRs form homodimers and bind to inverted repeats of HREs, while type-2 NRs bind to direct repeats of HREs and form heterodimers - most commonly with the retinoid X receptor (RXR).[37, 38] Type-3 NRs combine characteristics of type-1 and -2 by forming homodimers but binding to direct repeats of HREs, and type 4 NRs bind to half-site HREs as monomers.[39, 40] Furthermore, NRs differ in their mode of action as illustrated in Figure-2, showing the comparison of type-1 and type-2 effector function in either the cytoplasm or cell nucleus. Activation of type-1 NRs by their natural ligand occurs prior to dimerization in the cytoplasm

via the removal of a stabilizing heat shock protein (HSP) aiming straight for gene transcription in the cell nucleus.[41] Type-2 NRs initially form heterodimers and prevent the transcription of their target genes together with co-repressors in the cell nucleus.[37] The activation of type-2 NR-heterodimer complexes by their natural ligands results in a decoupling from the co-repressors in the cell nucleus, subsequently initiating the transcription process.[38, 42] Ultimately, co-activators support and trigger the DNA translation.[43] Lastly, NRs may also harbor non-genomic functions right after biosynthesis, which however are still yet not fully elucidated.[44]

3 Current and future NR based pharmacotherapeutics targeting liver fibrosis

As of today, there is no approved pharmacotherapy for liver fibrosis, neither NR based nor by any other pharmaceutical approach. Current therapies mainly focus on treating the underlying etiology, thus preventing disease progression by treating viral hepatitis, advocating alcohol abstinence or a change in life-style.[14, 15] Yet, slowing down fibrosis progression or even promoting fibrosis regression are desirable goals and thus in the focus of present research. Many associated mechanisms are currently being investigated, mainly focusing on cell stress, apoptosis, inflammation, metabolic pathways, HSC activation and ECM degradation.[2]

NRs in liver tissue mainly coordinate metabolic processes such as bile acid, lipid, and glucose homeostasis, positioning them as promising therapeutic targets in metabolic and cholestatic liver diseases. However, they also play a key role in the regulation of inflammation, liver regeneration, cell differentiation, and consequentially HSC activation and liver fibrosis.[45] In the following sections, the most promising and currently investigated NR as targets for NR modulators are described. Figure-3 summarizes the general and presumed beneficial effects in liver disease mediated by NR that extend beyond liver fibrosis. The effects of NR modulators in specific hepatic cells depend on their expression pattern. Figure-4 visualizes the NR expression pattern of specific liver cells in healthy human liver

(*www.proteinatlas.org)*. However, since most NR-related research is first performed in animal models, species-specific differences need to be highlighted which may explain controversial results from NR modulation in different animals and patients. Table-1 summarizes the available evidence on liver cell-specific NR expression patterns considering different settings (i.e., healthy vs. fibrotic livers) and controversial data reported in previous studies.

A detailed and cell-specific overview of the impact NR-modulation has on liver cells (predominantly on HSCs, but as well on KCs, HCs & liver sinusoidal endothelial cells (LSECs)) is presented in Figure-5 to highlight their role in the prevention and regression of liver fibrosis. Due to their promising therapeutic effects in liver diseases, many NR modulators are currently in clinical phase 2 and 3 studies as single or combination treatments, with liver fibrosis as primary or secondary readout. Due to their effects on the metabolic component of liver disease, most of them are currently considered as promising therapeutics primarily in non-alcoholic steatohepatitis (NASH) and Non-Alcoholic Fatty Liver Disease (NAFLD). Nevertheless, liver fibrosis regression/progression is usually included as side-parameter in these studies. Ongoing or recently completed clinical trials are summarized in Table-2 (data extracted from clinicaltrials.gov, 04.07.2021).

3.1 Farnesoid X Receptor

The Farnesoid X Receptor (FXR) mostly refers to FXR-α (NR1H4), since FXR-β (NR1H5) is a functional NR only in rodents and appears as nothing but a pseudogene in the human genome.[46, 47] FXR is the bile acid sensor of enterohepatic tissues and plays a crucial role in the maintenance of energy homeostasis by regulating the metabolism of bile acids, lipids and glucose.[48] Consequently, FXR has been mainly considered as target for cholestatic liver disease due to its major role in the direct and indirect regulation of bile acid synthesis.[49] FXR modulation also plays a critical role in NAFLD, owing to its comprehensive effects on lipid

and glucose metabolism.[50] This is reflected by FXR$^{-/-}$ mice, which developed spontaneous steatosis, hypertriglyceridemia and insulin resistance.[51] Additionally, FXR$^{-/-}$ mice were more susceptible to inflammatory stress, highlighting the anti-inflammatory properties of FXR.[27] In brief, FXR interacts with NF-κB signaling and inhibits pro-inflammatory genes (e.g., TNF-α, inducible nitric oxide synthase (iNOS), Cyclooxygeanse-2 (COX-2), CCL-2 and IL-1β)), while NF-κB activation reciprocally antagonizes FXR activity.[27, 52, 53] This offers an explanation why FXR$^{-/-}$ mice demonstrate an increased expression of pro-inflammatory cytokines, a resistance to apoptosis and an impairment of liver growth and regeneration.[54-56] Furthermore, FXR activity and expression has been reported to be decreased in fibrotic or injured liver tissue in humans, as well as in mice.[48] While FXR in liver tissue is primarily expressed in hepatocytes, it is just marginally expressed in human HSCs[48, 57] and was reported to be non-detectable at biologically significant levels in mouse HSCs.[57]

Yet, FXR modulation impacts on HSC activity, as shown by the fact that FXR agonists reduced HSC activation and decreased liver fibrosis in several animal models.[58-60] In vitro experiments demonstrated that HSCs exposed to an FXR ligand show a reduced expression of alpha-1 type 1 collagen (col1α1) and TGF-β and an upregulation of the nuclear receptor NR0B2 – the small heterodimer partner (SHP).[61] Fiorucci et al. investigated the role of SHP in HSC inhibition, concluding that upregulation of SHP might lead to the major anti-fibrotic activity of FXR.[61, 62] In a bile duct ligation animal model, the loss of SHP resulted in an increased sensitivity to liver damage and fibrosis.[63] Furthermore, the activation of the FXR-SHP cascade by FXR agonism seemed to protect against liver fibrosis in another bile duct ligation model.[61] The anti-fibrotic role of SHP was additionally confirmed in SHP-overexpressing LX2-HSCs by its counter-regulatory signal for HSCs transactivation, which prompted further studies on SHP signaling and identification of so far unknown SHP agonists.[64, 65] Further consequences of FXR activation include the downregulation of ECM

expression in HSCs and fibrogenesis via an increased mrR-29a promoter activity[66], and the inhibition of endothelin-1 (ET-1)[67], which would enhance pro-fibrogenic gene expression in HSCs.[68]

Nevertheless, the direct impact of FXR activation on liver fibrosis is still controversially discussed as there is partially conflicting data on FXR mRNA and protein expression in HSC and myofibrobasts.[57, 61] While Fiorucci et al. found FXR gene and protein expression in rat HSCs and in a passaged cell line HSC-T6[61], Fickert et al. reported only marginally expression levels of FXR in human HSCs and undetectable FXR protein expression in mouse HSCs.[57] This might be explained by interspecies differences but also requires further research to answer the question whether FXR activation occurs in HSCs and thus, directly induced anti-fibrotic effect in HSCs. Since antifibrotic effects of FXR agonists were constantly observed in several experimental studies[69, 70] and clinical trials,[71-73] HSC inhibition may also occur indirectly via paracellular anti-fibrotic molecular signals from other FXR-containing hepatic cells.

Next to the direct effects of FXR in liver tissue, the receptor contributes to inter-organ communication between gut and liver. Ileal FXR activation induces over-expression of fibroblast growth factor-19 (FGF-19; or its ortholog -15 in rodents) as a ligand to the FGF receptor-4 in the liver tissue via the enterohepatic signaling pathway, consequentially inhibiting the expression of CYP7A1 and thereby regulating bile acid homeostasis in a second pathway.[74] It is assumed that FGF-19/15 possesses anti-fibrotic effects, but further mechanistic explorations are still under investigation. FGF-15$^{-/-}$ mice fed with a high fat diet showed decreased liver fibrosis compared to wildtype animals[75] and a FGF19 analogue (M70/Aldafermin) resulted in anti-fibrotic activities in a mouse model of NASH.[76] However, Aldafermin (NGM282) did not reach statistical significance in histological improvement of liver fibrosis in NASH patients, even if a promising reduction in fibrosis stage and

biomarkers of fibrogenesis were reported at the clinical trials interim analysis.[77, 78] Although, Aldafermin showed significant reduction of fibrosis biomarkers Enhanced Liver Fibrosis (ELF) score and the pro-peptide of type 3 collagen (Pro-C3) level in primary sclerosing cholangitis (PSC) patients, still highlighting the role of FGF19 in liver disease and the FXR signaling cascade.[79]

An additional benefit of FXR activation in the intestine is based on intestinal immunity modulation and decreased ileal bacterial invasion.[80, 81] Overall, the gastrointestinal inflammatory response is reduced upon FXR activation, a phenomenon demonstrated in a cirrhotic rat model study of the FXR agonist INT-747, nowadays called obeticholic acid (OCA).[82] OCA led to a reduction in hepatic inflammation via an increased CYP450 expression in a high-fat-diet (HFD) mouse model. This is further supported by data of another FXR agonist (GW4064), which was linked to an amelioration of inflammation via the inhibition of TLR-4 in a model of lipopolysaccharide induced hepatic inflammation.[83, 84] GW4064 additionally decreased cytokine-STAT3 signaling, which is key in the protection of hepatocellular inflammation and the inhibition of NF-κB.[85] OCA, as steroidal FXR agonist, was and is still involved in various studies regarding the treatment liver disease, but unfortunately causes side effects such as pruritus. Furthermore, OCA and the improved FXR/TGR5 agonist INT-7667, induced an in-vitro dose-dependent reduction of collagen and increased MMP2-9 activity,[86] and WAY-362450 (FXR-450) hampered hepatic inflammation and fibrosis shown by a reduced expression of hepatic genes linked to fibrosis in a methionine choline-deficient (MCD) diet NASH model.[87] Importantly, OCA led to a small but significant reduction in fibrosis in NAFLD patients.[71]

Currently, new non-steroidal FXR agonists are under investigation, which show a higher degree of receptor specificity and selectivity and do not undergo enterohepatic circulation.[88, 89] PX20606 showed anti-fibrotic effects paired with a decrease in intestinal inflammation and

bacterial translocation in a carbon tetrachloride (CCl_4) rat model[69] and led to the development of Cilofexor (GS-9674) – another non-steroidal FXR agonist. Cilofexor was associated with a decrease in portal hypertension (what highlights FXR also as a vascular target[90]), reduced liver fibrosis in NASH rats and decreased mesenteric hyperperfusion in combination with propranolol.[70] In NASH patients, Cilofexor showed partly reduction of multiple markers of fibrosis as ELF components and liver stiffness by transient elastography.[91] Cilofexor combined as treatment with a liver-directed acetyl-CoA carboxylase (ACC) inhibitor (Firsocostat - ND-630) even led to significant decrease of NASH Clinical Research Network (CRN) fibrosis score and a beneficial shift of histological liver fibrosis score in NASH patients.[92] Still, Cilofexor needs to be investigated as single and combination treatment in further large-scaled and long-term studies.

FXR acts as transcription factor on several metabolic processes and targets multiple hepatic pathways. Thus, FXR agonists are one of the most important, promising and widely represented NR modulators that might be useful in the treatment of liver disease of several etiologies. Whilst the most promising results have been shown in metabolic liver diseases such as NALFD and NASH, future insights on their anti-fibrotic effects might give rise to combination therapies against liver fibrosis in general.

3.2 Vitamin D Receptor

The natural ligand of the Vitamin D receptor (VDR – NR1I1)) is 1,25(OH)(2)D(3), the active form of vitamin D. Similar to many other NRs discussed in this review, VDR undergoes a conformational rearrangement and forms a heterodimer with RXR.[93] Even though the overall expression of VDR in the hepatic tissue is low, non-parenchymal cells such as HSCs (mainly responsible for liver fibrosis) and biliary epithelial cells show high expression.[94] In an animal study, $VDR^{-/-}$ mice developed spontaneous liver fibrosis, thus indicating the importance of VDR in liver fibrosis and showing that VDR signaling regulates the inhibition of HSC

activation.[95] Briefly, VDR activation inhibits the signaling of a TGF-β/SMAD-dependent transcription response in HSCs.[95] The substitution of 1,25(OH)(2)D(3) and its protective effects against liver injury were demonstrated in a rodent thioacetamide (TAA) model.[96] The same research group claimed that the application of 1,25(OH)(2)D(3) inhibits the development of liver fibrosis, but cannot ameliorate established cirrhosis, as shown in another animal model.[97] Results from other studies further support the protective role of Vitamin D and VDR activation against hepatic fibrosis, evidenced by a reduction of hydroxyproline levels and decreased expression levels of profibrogenic genes (e.g., col1α1 and tissue inhibitor of metalloproteinase-1 (TIMP-1)) in Mdr2$^{-/-}$ mice after supplementation of Vitamin D.[98] In humans, Vitamin D deficiency seems to be highly prevalent in patients with liver fibrosis. Yet calcitriol supplementation is not part of the clinical routine owing to a short half-life and possible hypercalcemia.[99, 100] Furthermore, several VDR gene polymorphisms (e.g., in HCV patients) were identified as important determinants of Vit D production. However, further large scale prospective cohort studies are needed in order to elucidate the safety and efficacy of Vit D supplementation or VDR activation in the setting of liver fibrosis and to investigate potential effects on HSC activation.[99, 101-103] Currently, VDR activation by 1,25(OH)(2)D(3) does not seem to be an effective treatment option in patients with liver fibrosis and Vitamin D will only be administered as a supplemental treatment.

3.3 Peroxisome Proliferator-Activated Receptor

Peroxisome proliferator-activated receptors (PPARs) represent a promising pharmacological target in liver disease, especially in NAFLD. PPARs include three distinct isoforms: PPAR-α (NR1C1), which is highly expressed in oxidative tissues such as in liver. PPAR-δ (NR1C2), which is expressed in inflammatory cells and different liver cells including hepatocytes, HCSs and Kupffer cells. And PPAR-γ (NR1C3), traded as the most promising PPAR target involved in HSC activation.[104] Furthermore, PPAR-γ is commonly expressed in macrophages

and yields anti-inflammatory effects in liver fibrosis.[105] The different PPAR isoforms induce similar downstream effects and function such as regulators of energy homeostasis by influencing lipid and glucose metabolism. PPARs form a heterodimer with RXR after activation by their natural ligands, fatty acids and eicosansoids. Due to differences in their tissue/cellular expression level, PPARs may exert distinct effects in regard to liver fibrosis in different types of liver cells.[106, 107] PPARs have been reported to be decreased in liver cirrhosis, as shown by Boyer-Diaz et al. when comparing hepatic PPAR expression in healthy liver tissue vs. cirrhotic liver tissue from patients with alcohol related liver disease (ALD) or NASH.[108] The expression pattern of PPARs in rodent animal models comparing healthy rats to TAA and BDL rats highlighted differences related to type of liver injury/disease or species. While all PPAR subtypes were shown to be downregulated in patients with ALD/NASH and TAA rats compared to healthy liver tissue, PPAR-δ was upregulated in BDL rats;[108] which would explain some controversial data on the hepatic PPAR expression patterns. The study of Boyer-Diaz et al. also provided insight into the cell-specific expression pattern of PPAR-α/-δ/-γ in the TAA model; where a differential regulation of PPAR subtypes in different liver cell types was demonstrated. Importantly, these results on differential expression of PPAR isoforms from distinct cell types may explain differences to studies assessing PPAR expression in bulk liver tissue only. Briefly, the PPAR subtypes were all decreased accept of upregulation of PPAR-α in LSECs, PPAR-γ in HCs/HSCs and PPAR-δ in LSECs/KCs.[108] Further studies on liver tissue of different fibrosis stages are needed to extend the knowledge about the regulation of PPAR expression patterns during disease progression. This would also allow to tailor the design of specific single and pan-PAAR-agonists to the specific liver disease etiology and disease stage.

3.3.1 PPAR-α

PPAR-α is involved in the regulation of pro-inflammatory genes, mainly by limiting cytokine

expression.[109] PPAR-α directly binds to inflammatory transcription factors in hepatocytes such as p65 & c-Jun (NF-κB components), AP-1, STAT and thereby suppresses their transcriptional activity.[110] In Kupffer cells, PPAR-α activation is linked to the downregulation of IL-15 and IL-18, as shown by a study of macrophage-specific PPAR-α-deficient mice.[111] This indicates that Kupffer cell PPAR-α activation mediates anti-inflammatory effects by possible prevention of macrophage polarization.[111] In HSCs, however, the role of PPAR-α is still poorly characterized, even though anti-fibrotic effects of the PPAR-α agonist Wy14643 have previously been demonstrated in murine models of liver fibrosis.[112, 113] Briefly, in rodent CCl_4 or thioacetamide models, the PPAR-α ligand Wy14643 has been shown to decrease liver fibrosis and HSC activation, reduce liver steatosis and even reverse histological liver fibrosis.[112, 114] An additional study on oleoylethanolamide in methionine choline-deficient diet and thioacetamide murine mouse model indicated that TGF-β stimulation may be inhibited by endogenous PPAR-α ligands, thus inhibiting HSC activation.[115] Next to this, PPAR-α seems to be protect endothelial function, which might ameliorate intrahepatic portal hypertension.[116-118]

Whilst all these studies may give a first insight into the role of PPAR-α in liver disease, further mechanistic studies are required to decipher all potential anti-fibrotic effects. Yet, synthetic PPAR-α ligands are already in clinical use for the treatment of hypertriglyceridemia. Furthermore, controversially documented inter-species differences in PPAR-α expression[119-121] complicate the translation of data from preclinical studies to patients.[122 123]

3.3.2 PPAR-γ

PPAR-γ represents the most promising PPAR target in HSCs, since it is a key factor in HSCs activation and phenotype alteration.[104] The presumed equilibrium between PPAR-γ expression in HSCs and HSC activation, is mainly mediated by TNF-α, inhibiting PPAR-γ

activity at a posttranslational level.[124, 125] HSC activation is therefore associated with low levels of PPAR-γ expression, and the assumed relation between PPAR-γ and liver fibrosis was confirmed in an experimental restoration of PPAR-γ levels in HSCs, which resulted in a regression of aHSCs to quiescence state.[126, 127] Furthermore, PPAR-γ prevents the TGF-β/SMAD pathway in pro-fibrotic MFs.[128, 129] The change of HSC phenotype was further investigated in different mouse models of induced liver fibrosis (CCl_4/dimethylnitrosamine intoxication and bile duct ligation) in which the synthetic PPAR-γ ligand thiazolidinedione was linked to a reduction of ECM deposition and HSC activation.[130] Several other in vitro[131] and in vivo studies[132, 133] on HSCs confirmed the anti-fibrotic potential of synthetic PPAR-γ ligands. Interestingly, in vitro studies investigating PPAR-γ activation in HSCs showed an additional decrease of HSC proliferation after PDGF-induction.[129, 134] Thus, PPAR-γ activation might affect the PDGF transduction signal by inhibiting extracellular factor-regulated kinase (ERK) activity.[135] Even a cross-pathway to FXR has been described in previous literature: PPAR-γ seems to be positively modulated by FXR via SHP and counter-regulates the pro-inflammatory phenotype of HSCs – another benefit of the FXR/SHP pathway.[64] Next to its role in HSCs, PPAR-γ is primarily expressed in macrophages and is capable to inhibit AP-1, STAT1 and NF-κB, therefore decreasing the inflammatory response.[136] The regulation of hepatic inflammation by PPAR-γ was confirmed in a CCl_4 mouse model study[137], and the PPAR-γ agonist Pioglitazone was even shown to reduce hepatic fibrosis in a CCl_4 and choline deficient diet model and decrease pro-fibrotic gene expression (e.g., col1α1, αSma).[138] In contrast to these findings, the same PPAR-γ agonist failed to achieve beneficial results in a bile duct ligation model.[138]

Likewise, treatment with Saroglitazar (dual PPAR-α/-γ agonist) was associated with a significant decrease of liver collagen content, cholangiocyte proliferation marker (CK19) and several fibrosis markers (col1α1 & αSma) in a $Mdr2^{-/-}$ mice models of primary sclerosing

cholangitis.[139] As of today, Saroglitazar is still being investigated as treatment option for NASH patients and recently completed phase 2 studies, which showed a dose dependent improvement in the serum lipid profile and atherogenic lipoproteins as well as signs of a possible anti-fibrotic effect.[140]

Overall, the promising effects of PPAR-γ modulation led to the investigation of PPAR-γ agonist in several clinical trials regarding liver diseases, showing antifibrotic effects in NASH.

3.3.3 PPAR-δ

As compared to PPAR-α and -γ, the expression of PPAR-δ is particularly high in HSCs, especially in aHSCs.[141] Deviating PPAR-δ expression levels might arise from the altered Vit A status upon HSC activation, since PPAR-δ regulates vitamin A metabolism-related gene expressions.[29, 141] However, the influence of PPAR-δ modulation on HSCs and a possible subsequent impact on liver fibrosis has not been fully investigated yet.[142] PPAR-$\delta^{-/-}$ mice showed a reduction in adiposity compared to wild-type animals, which revealed that PPAR-δ is highly involved in the systemic lipid metabolism[143] and regulation of serum/hepatic triglyceride levels[144, 145], thus highlighting their potential as therapeutic option in metabolic liver diseases. PPAR-δ induce monounsaturated fatty acids, which in turn prompts KCs to modulate the immune response via a reduction of TNF-α or IFN-α.[144] By this, e.g. the PPAR-δ agonist GW0742 was able to reduce hepatoxicity by downregulating the expression of proinflammatory genes in a CCl_4 mouse model via the modulation of NF-κB signaling.[146] Another study using the PPAR-δ agonist KD3010 demonstrated an amelioration of liver injury in a CCl_4 mouse model, which was confirmed by a study showing further anti-fibrotic effects after bile duct ligation in mice.[147] Contrary to this, PPAR-δ agonists, such as L165041, increased hepatic stellate cell proliferation during inflammation in rats[148] and the PPAR-δ ligand GW501516 was linked to enhanced fibrotic and inflammatory responses due

to an increased phosphorylation of p38 and c-Jun-N-terminal kinases.[149]

The reason for these contradictory effects might arise due to different PPAR-δ agonists, again highlighting the need for further studies to fully elucidate the role of PPAR-δ in liver fibrosis. Currently, PPAR-δ agonists have been insufficiently characterized in clinical studies and were mostly tested as single PPAR-δ agonists in experimental animal models, where they demonstrated almost no impact on liver injury improvement, shifting the focus on dyslipidemia studies or studies regarding metabolic disorders.[147] In human studies, they usually appear in dual & pan-PPAR therapies.

3.3.4 Dual & pan-PPAR therapy

PPAR-δ agonists are mostly tested in patients as dual agonists to combine the positive effects. For example, the dual PPAR-α/δ agonist Elafibranor (GFT505) showed promising effects in preclinical studies.[150] The administration of Elafibranor was linked to a beneficial regulation of lipid metabolism, fatty acid transport and oxidation, as well as positive changes in glucose metabolism and inflammation.[151, 152] Elafibranor is currently investigated as a treatment option for primary biliary cirrhosis (PBC) patients in a clinical phase 3 study (NCT04526665) after a positive phase 2 trial.[153] Unfortunately, two previous clinical trials in NASH patients had to be terminated, since Elafibranor was unable to hit the primary objective of resolving NASH without worsening liver fibrosis compared to a placebo (NCT03883607 & NCT02704403). Another pan-PPAR agonist Lanifibranor (IVA-337) was recently investigated and did not only show significantly increased high density lipoprotein cholesterol (HDL-C) and decreased triglycerides levels, but also led to the resolution of NASH and regression of fibrosis.[154] The treatment of PBC patients seems as well promising by administration of another pan-PPAR agonist (Bezafibrate - even if predominantly for PPAR-α) in combination with ursodeoxycholic acid therapy, showing anti-inflammatory, anti-fibrotic and anti-cholestatic effects.[155]

Although most single, dual or pan-PPAR agonists demonstrated beneficial effects on liver fibrosis, their efficacy in patients is still incompletely assessed or not proven by histology and needs further late-stage clinical trials. Novel and more selective PPAR agonists might minimize adverse effects of current agonists and target liver fibrosis more specifically. Especially the investigation of pan-PPAR agonists, targeting all three PPAR isoforms emerged as promising therapeutic strategy.

3.4 Liver X Receptor

The Liver X Receptor (LXR) includes two different isoforms: LXR-α (NR1H3) and LXR-β (NR1H2).[156] While their role in cholesterol metabolism and hepatic steatosis is known quite well, they may also be involved in the regulation of hepatic inflammation and fibrosis.[157] Beaven et al. showed the role of LXR in an elegant in vitro and in vivo settings with $LXR^{-/-}$ animals:[158] $LXR^{-/-}$ primary isolated HSCs showed increased col1α1 and other pro-inflammatory gene expression (e.g., PDGF-β, Acta2 and CCL-2) compared to wildtype animals. Furthermore, exposition of $LXR^{-/-}$ animals to CCl_4 or methionine choline deficiency diet led to increased αSma and collagen proportionate area, respectively. Subsequently, the stimulation of LXR in wildtype HSCs resulted in decreased expression levels of CCL-2 and IL-6 next to a suppression of col1α1 and Acta gene expression. Data presented by Beaven et al. highlighted the pivotal role of LXR-β in liver fibrosis and HSC activation.[158]

LXR-α activation in HCs mainly activates lipogenesis and bile acid export and LXR-β is predominantly expressed in HSCs, but an increase of LXR-α expression has been detected in qHSCs.[159] The role of either LXR-α or -β in KCs is still widely unknown, even though it has been shown that the activation of LXR in KCs suppresses the release of inflammatory mediators.[160] Direct LXR target genes are ATP binding cassette subfamily A member-1 (ABCA-1) which maintains reverse cholesterol transport and inhibits TLR-2, -4 and -9, NF-κB as well as mitogen-activated protein kinases (MAPK) signaling in macrophages.[161, 162]

Via this pathway, LXR activation e.g. attenuates the LPS-induced expression of pro-inflammatory molecules and inhibits pro-fibrotic pathways, mainly by inhibition of NF-κB.[163] Hence, LXRs have emerged as important regulators of innate immunity and several studies have shown that LXRs contribute to liver fibrosis via HSC activation. This resulted in the investigation of LXR modulators as potential therapeutic strategy against liver disease.

Whilst LXR activation seems to have the potential to ameliorate liver fibrosis and despite promising results in animal studies, LXR pan-modulators were linked to side effects such as hyperlipidemia and liver steatosis. Since these side effects are primarily assumed to be caused by LXR-α, further research on partial LXR-β agonism is warranted.[164] Synthetic non-specific LXR agonist, such as T0901317, were even able to promote a redifferentiation of primary LSECs in liver diseased animal models.[165] LSECs lose their phenotype and protective properties along with liver injury, which is paralleled by vasoconstriction and angiogenesis.[166] LSECs are key to maintain liver homeostasis and preserving their initial phenotype or re-differentiate their phenotype represents a novel approach in liver fibrosis regression.[166] Paradoxically, the LXR inverse agonist SR9243 had similar beneficial anti-fibrotic and anti-inflammatory effects in two BASH (both-alcoholic and steatohepatitis) mouse models, which were induced by using a high-cholesterol diet in combination with either CCl_4 administration or bile-duct ligation.[167] Moreover, the inhibition of LXR activation by the synthetic inhibitor SR928 showed a significant improvement in liver fibrosis severity in a mouse NASH model (high-trans-fat, fructose and cholesterol diet induced ob/ob mouse).[168] All these studies support the pivotal role of LXR in liver fibrosis. However, the anti-fibrotic effect of LXR modulators are controversial and still under investigation.

3.5 Retinoid X receptor/Retinoic acid receptor

The retinoid X receptor (RXR) usually forms heterodimers with several other NRs (e.g.,

FXR, PPAR, LXR) and thereby has broad implications for fibrogenesis.[169] HSCs express three isoforms of RXR: -α, -β and -γ, [170], with RXR-α (NR2B1) being the dominant isoform in cultured HSCs.[171] While 9-cis retinoic acid (9cRA) is the natural ligand of RXR, some studies demonstrated that all-trans retinoic acids (atRAs) might bind to RXR.[172] atRAs are the natural ligands of the retinoid acid receptor (RAR), which forms a heterodimer with RXR.

Since HSCs function as a central storage of retinoids, RXR and RAR might be involved in the activation of HSC.[170] Still, the role of RXR activation is controversially discussed[173] and in vivo studies suggested that 9-cis retinoic acid exacerbated rat liver fibrosis by inducing the activation of TGF-β1.[174, 175] Ye et al. even suggested that RXR modulation treatment might be a dose-dependent issue via the inhibition of TGF-β1.[176] Additionally, Hellemans et al. showed a difference in the phenotype of HSCs depending on the presence of natural or synthetic retinoids, which might explain divergent results by prior studies.[177] The activation of RAR in HSCs, specifically RAR-β (NR1B2), results in a downregulation of myosin light chain-2 (MLC-2) expression, which plays a pivotal role in ECM deposition.[178] Furthermore, the administration of atRAs in vitro to HSCs and in vivo in cholestatic animal models was linked to an inhibition of pro-fibrotic gene expression in (TGF-β, col1α1, αSMA, and MMP-2)[179-182] and showed protective properties in regard to ECM accumulation.[183-185] The role of RAR in aHSCs was further elucidated by data of a synthetic RAR antagonists, which initiated TGF-β dependent procollagen synthesis and mitogenesis in HSCs.[186] Overall, the critical role of RAR/RXR in HCS activation is well established, yet further research is needed to determine precise mechanisms. Particularly the role of RXR modulators in liver fibrosis is still not sufficiently researched, partly owing to complex heterodimer interactions with retinoic acid receptors (NR1B) as well as the retinoic acid response element as a potentially shared target. In general, the fact that RXR acts as heterodimer-partner for several other NRs and neither RAR nor RXR are able to respond to agonists unless a ligand is bound to the

heterodimeric partner, increases the complexity of RXR research.[187]

3.7 Other evidence for nuclear receptor modulators as treatment options for liver fibrosis

Thyroid hormone receptors (THRs) might function as possible targets for anti-fibrotic therapeutics, even though it is primarily involved in the metabolism of cholesterol and lipoproteins.[188] Current research focuses on liver tissue specific THR-β activators, since they result in an increased cholesterol uptake and synthesis in hepatocytes, thereby providing an attractive option in the treatment of NAFLD and NASH. However, unspecific THR activation has been shown to be detrimental, as an activation of the THR-α receptor may lead to cardiotoxicity.[189] Two promising therapeutic THR-β modulators are currently examined in phase 2 and 3 studies for NASH and NAFLD (NCT04173065, NCT04197479 & NCT03900429). Interestingly, NAFLD progression was associated with hypothyroidism, whereas hyperthyroidism appeared to slow down fibrotic remodeling in the liver of NAFLD patients.[190, 191] This finding is supported by in-vitro evidence, showing that THR-β and THR-α expression is repressed during liver injury in PBC and NASH patients and that THR-α is predominantly expressed in HSCs and might be involved in HSC cell differentiation.[192] THR-α seems to be involved in the fibrogenic response of HSCs via the TGF-β pathway, which might be interesting for future therapeutic approaches, particularly when considering all disadvantages of THR-α activation.

The pregnane X receptor (PXR) agonist is also seen as a potential target for anti-fibrotic therapy. It was associated with an inhibition of fibrogenesis in rodent animal models via PXR dependent and independent pathways.[193] Moreover, experiments with invitro PXR activation decreased cell differentiation of human HSCs.[194]

Another approach might to target glucocorticoid receptors (GR) in combination treatments. GR activation decreases TGF-β signaling, hence impacting the phenotype of HSCs.[195, 196]

While the treatment with glucocorticoids shows opposing effects on HSCs and immune cells, Kim et al. suggested that the impact of GR activation is likely mediated by repressing NF-κB, SMAD3 and AP-1, thus demonstrating anti-inflammatory and possibly anti-fibrotic effects.[197] After Koorneef et al. recently found that selective GR modulation prevents and reverses NASH in a mouse model[198] the impact of glucocorticoids in NASH is currently the topic of a clinical study (NCT03823703).

Targeting androgen receptors (ARs) with bioidentical testosterone such as the agonist LPCN 1144, offered treatment potential in NASH as shown in a rabbit NASH model in which the percentage of fibrosis was improved upon AR activation.[199] LPCN 1144 is currently under evaluation in regard to efficacy, safety, and tolerability in NASH patients (NCT04134091). Finally, Estrogen receptors (ER) need to be mentioned, even though the use of ER activators in liver disease is controversially discussed. However, the beneficial anti-fibrotic effect of ER activators in the hepatic tissue (e.g. 17b-estradiol and estradiol)[200-202] may also explain sex differences in liver fibrosis and cirrhosis progression.[203-206]

4. Conclusions for potential therapeutic applications of nuclear receptor modulators in liver fibrosis:

NRs are able to directly modulate hepatic gene expression, either via naturally occurring or synthetic modulators, thus, offering a targeted approach to influence HSC activation, inflammation and fibrogenesis. Research in knock-out mouse models and experimental modulations of NRs broadened the knowledge on anti-fibrotic and cell specific effects in vitro and in vivo. Still, further research on NR modulators is warranted in order to investigate their complex molecular signals and regulatory function in different types of liver disease. Even though certain NRs represent promising therapeutic targets in liver fibrosis, most NR modulators being under clinical investigation exert pronounced metabolic effects and thus may most suitable for metabolic dysfunction-associated liver diseases (i.e., NAFLD and

NASH). Importantly, future research should try to decipher the indirect impact of NRs on liver fibrosis versus their indirect anti-fibrotic effects. Many pharmacologic modulators of NRs have shown promising anti-inflammatory and anti-fibrotic effects in preclinical and early clinical studies, however, limited results on convincing fibrosis-related clinical endpoints have been reported. Some NR modulators were associated with dose-limiting side effects and, consequently may rather be attractive combination compounds for other anti-fibrotic therapeutics. While clinical trials including fibrosis-related endpoints of single and combination NR treatments are ongoing, basic and experimental research is warranted to further decipher molecular signals of NR modulation on the single cell level across different types and stages of liver disease in order to better understand their therapeutic potential.

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Abbreviations:

ACC	Acetyl-CoA Carboxylase
AF	Activation Factor
aHSC	activated Hepatic Stellate Cell
ALD	alcohol-related liver disease
AP-1	Activator Protein 1
AR	Androgen Receptor
αSMA	α Smooth Muscle Actin

atRAs	All-Trans-Retinoic Acids
BASH	Both-Alcoholic and Steatohepatitis
BECs	Biliary Epithelial Cells
CCL	Chemokine CC-motif Ligand
CCl_4	Carbon Tetrachloride
CCRn	C-C chemokine receptor type n
col1α1	Alpha-1 Type 1 Collagen
COX-2	Cyclooxygeanse-2
DBD	DNA Binding Domain
ECM	Extracellular Matrix
ELF	Enhanced Liver Fibrosis
EMT	epithelial-to-mesenchymal transition
EndoMT	endothelial-to-mesenchymal transition
ER	Estrogen receptors
ERK	Extracellular Factor-Regulated Kinase
ET-1	Endothelin 1
FGF	Fibroblast Growth Factor
FXR	Farnesoid X Receptor
GLP-1	Glucocorticoid receptor-1
GR	Glucocorticoid Receptor
HC	Hepatocyte
HCV	Hepatitis C Virus
HDL-C	High Density Lipoprotein Cholesterol
HFD	High Fat Diet
HRE	Hormone Response Element
HSC	Hepatic Stellate Cell
HSP	Heat Shock Protein
ICAM1	Intercellular Adhesion Molecule 1
IL	Interleukin
INF	Interferon
iNOS	inducible Nitric Oxide Synthase
IκBα	inhibitor of nuclear factor kappa B
KC	Kupfer Cell
LBD	Ligand Binding Domain
LSEC	Liver sinusoidal endothelial cells
LXR	Liver X Receptor
MAPK	Mitogen Activated Protein Kinases
MCD	Methionine Choline Deficient
MF	Myofibroblast
MLC-2	Myosin Light Chain-2
MMP	Matrix Metalloprotease
MR	Mineralcorticoid receptor
MSCP-1	Mitochondrial Substrate Carrier Protein-1
NAFLD	Non-Alcoholic Fatty Liver Disease

NASH CRN	NASH Clinical Research Network
NASH	Non-Alcoholic Steatohepatitis
NF-κB	Nuclear Factor 'kappa-light-chain-enhancer' of activated B-cells
NR	Nuclear Receptor
NTD	N-Terminal Domain
OCA	Obeticholic Acid
PBC	Primary Biliary Cirrhosis
PDGF	Platelet-Derived Growth Factor
PPAR	Peroxisome Proliferator Activated Receptor
Pro-C3	Pro-Peptide of Type 3 Collagen
PSC	Primary Sclerosing Cholangitis
PXR	Pregane X Receptor
qHSC	quiescent Hepatic Stellate Cell
RAR	Retinoid Acid Receptor
RXR	Retinoid X Receptor
SGLT	Sodium glucose transport proteins
SHP	Small Heterodimer Partner
SMAD	Mothers Against Decapentaplegic Homolog
STAT	Signal Transducer and Activator of Transcription
TAA	Thioacetamide
TFRE	Transcription Factor Response Element
TGF-β	Transforming Growth Factor β
TGR5	G-Protein-coupled Bile Acid Receptor 5
THR	Thyroid Hormone Receptor
TIMP-1	Tissue Inhibitor of Metalloproteinase-1
TLR	Toll-Like Receptor
TNF-α	Tumor Necrosis Factor α
VDR	Vitamin D Receptor

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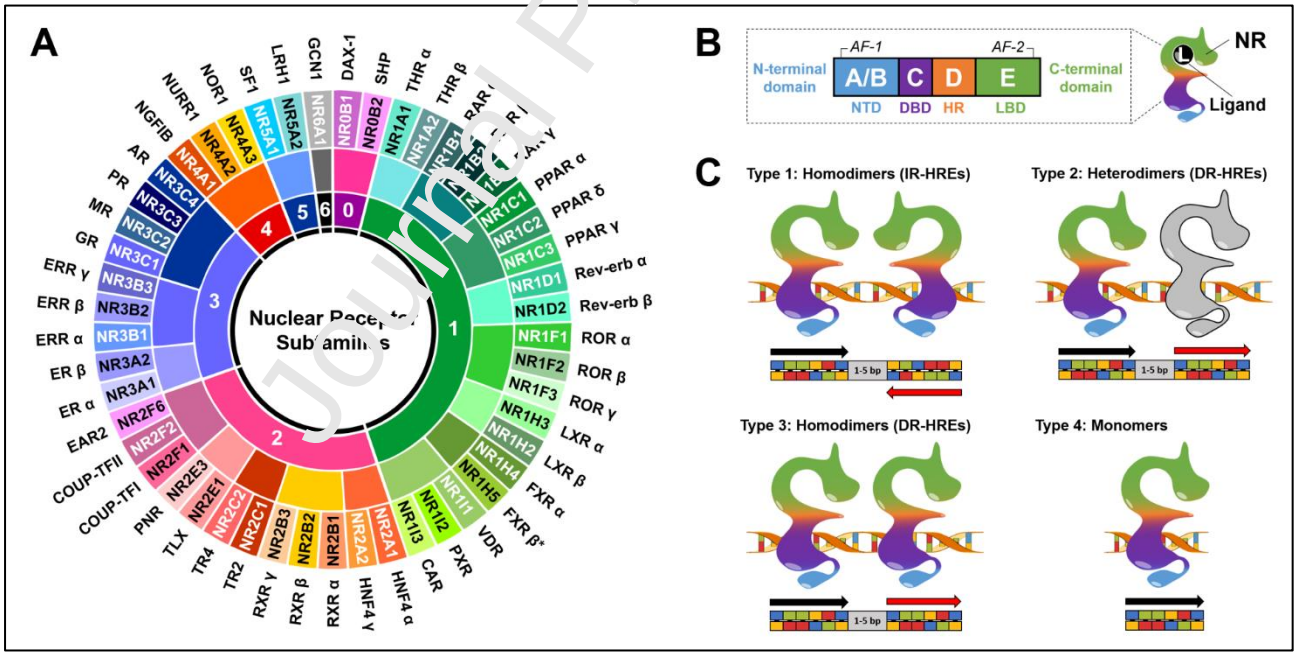

FIGURE 1. (A) Overview and classification of nuclear receptors by subfamilies: A0: Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene-1 (DAX-1), Small heterodimer partner (SHP), A1: Thyroid hormone receptor-α & -β (THR-α & -β), Retinoic acid receptor-α, -β & -γ (RAR-α, -β & -γ), Peroxisome proliferator-activated receptor-α, -β & -γ (PPAR-α, -β & -γ), Rev-erb-α & -β, RAR-related orphan receptor-α, -β & -γ (ROR-α, -β & -γ), Liver X receptor-α & -β (LXR-α & -β), Farnesoid X receptor-α (FXR-α), Farnesoid X receptor-β (FXR-β; *pseudogene in human), Vitamin D

receptor (VDR), Pregnane X receptor (PXR), Constitutive androstane receptor (CAR), A2: Hepatocyte nuclear factor-4-α & - γ (HNF4-α & -γ), Retinoid X receptor-α, -β & -γ (RXR-α, -β & -γ), Testicular receptor-2 & -4 (TR-2 & -4), Homologue of the Drosophila tailless gene (TLX), Photoreceptor cell-specific nuclear receptor (PRN), Chicken ovalbumin upstream promoter-transcription factor-I & -II (COUP-TF-I & -II), V-erbA-related protein-2 (EAR-2), A3: Estrogen receptor-α & -β (ER-α & -β), Estrogen-related receptor-α, -β & -γ (ERR-α, -β & -γ), Glucocorticoid receptor (GR), Mineralocorticoid receptor (MR), Progesterone receptor (PR), Androgen receptor (AR), A4: Nerve Growth factor IB (NGFIB), Nuclear receptor related protein-1 (NURR-1), Neuron-derived orphan receptor-1 (NOR-1), A5: Steroidogenic factor-1 (SF-1), Liver receptor homolog-1 (LRH-1), A6: Germ cell nuclear factor protein-1 (GCN-1). **(B)** Domains and components of nuclear receptors: activation factor-1 (AF-1), N-terminal domain (NTD), DNA binding domain (DBD), flexible hinge region (HR), C-terminal ligand binding domain (LBD) and activation factor-2 (AF-2) **(C)** NR-classification based on dimerization & binding type to hormone response elements (HREs): Type 1: Homodimers binding to inverted repeats of HREs spaced by 1-5 bp (IR-HREs); Type 2: Heterodimers binding to direct repeats of HREs spaced by 1-5 bp (DR-HREs); Type 3: Homodimers binding to direct repeats spaced by 1-5 bp (DR-HREs); and Type 4: Monomers binding to single HREs.

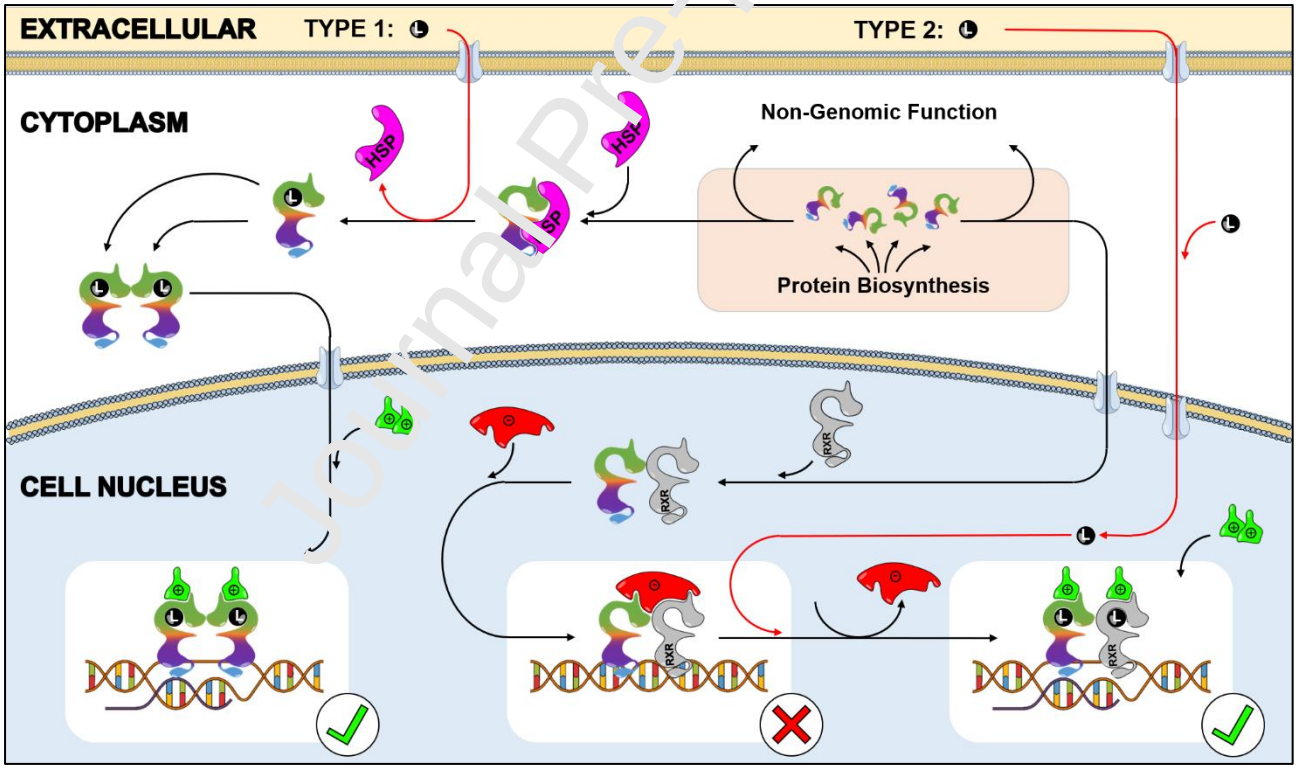

FIGURE 2. Different types of nuclear receptor effector function. Type-1 response of NRs is mediated by ligand-induced removal of stabilizing HSP from nuclear ligand, which can subsequently dimerize and translocate to the cell nucleus, where transcription of response elements is initiated and supported by co-activators. Type-2 response of NR requires the ligand interacting with the inactive NR inside the cell nucleus, where it induces activation of the already heterodimerized NR (mainly with RXR) to initiate transcription by replacement of co-repressors and the help of co-activators.

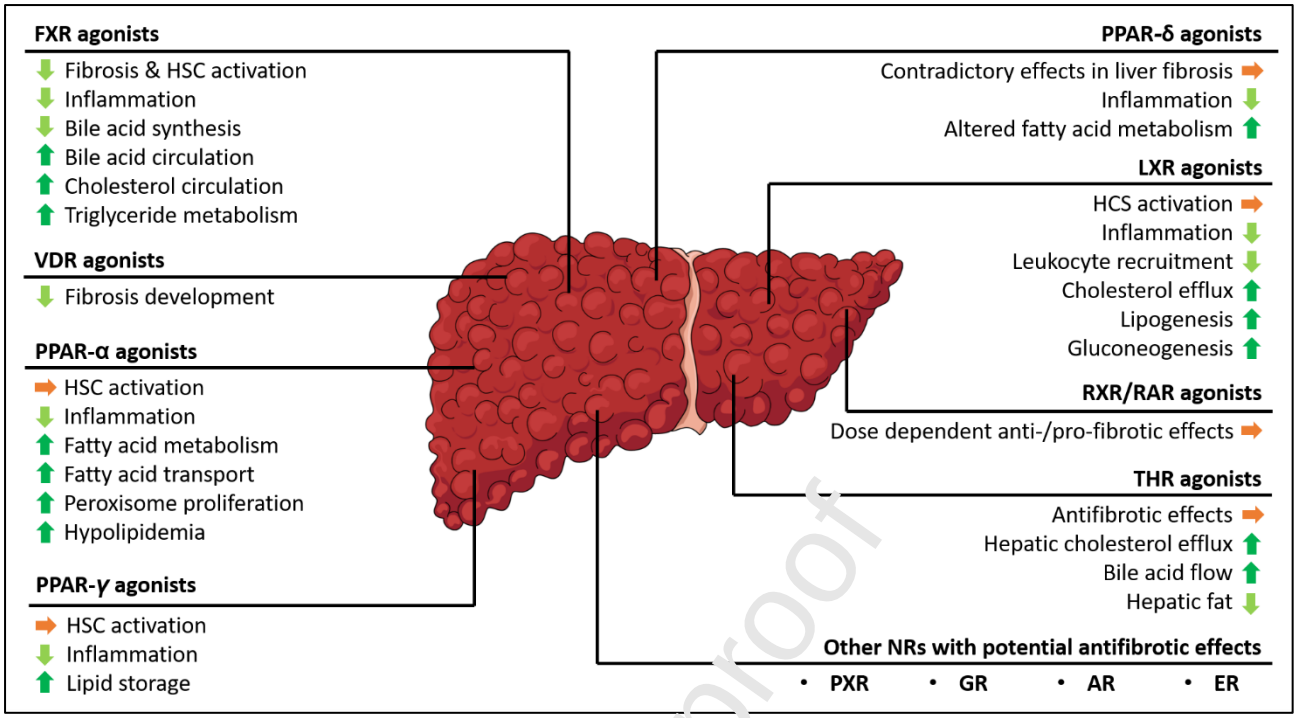

FIGURE 3. Overview on the reported effects of NR modulations as potential therapeutic approaches against liver diseases. Arrows indicate the presumed beneficial effects in liver disease by up- (dark green) or down- (light green) regulation of specific mechanisms that were reported to support liver fibrosis regression or to reduce liver fibrosis. Orange arrows indicate effects and mechanisms of NR modulation that are either controversial or yet insufficiently assessed.

NR	Subtype	Gene	HCs	HSCs	LSECs	BECs	KCs	T-Cells
FXR	FXR-α	NR1H4						
	FXR-β	NR1H5						
VDR	VDR	NR1I1						
PPAR	PPAR-α	NR1C1						
	PPAR-γ	NR1C3						
	PPAR-δ	NR1C2						
LXR	LXR-α	NR1H3						
	LXR-β	NR1H2						
RXR	RXR-α	NR2B1						
	RXR-β	NR2B2						
	RXR-γ	NR2B3						
RAR	RAR-α	NR1B1						
	RAR-β	NR1B2						
	RAR-γ	NR1B3						
THR	THR-α	NR1A1						
	THR-β	NR1A2						

pTPM	0	>0-5	>5-10	>10-25	>25-50	>50

FIGURE 4. Protein expression of NRs in different cells of the healthy human liver: Hepatocytes (HCs), Hepatic stellate cells (HSCs), Liver sinusoidal cells (LSECs), Biliary endothelial cells (BECs), Kupfer cells (KCs) and T-Cells. (Data extracted from

www.proteinatlas.org accessed on 26 June-2021)

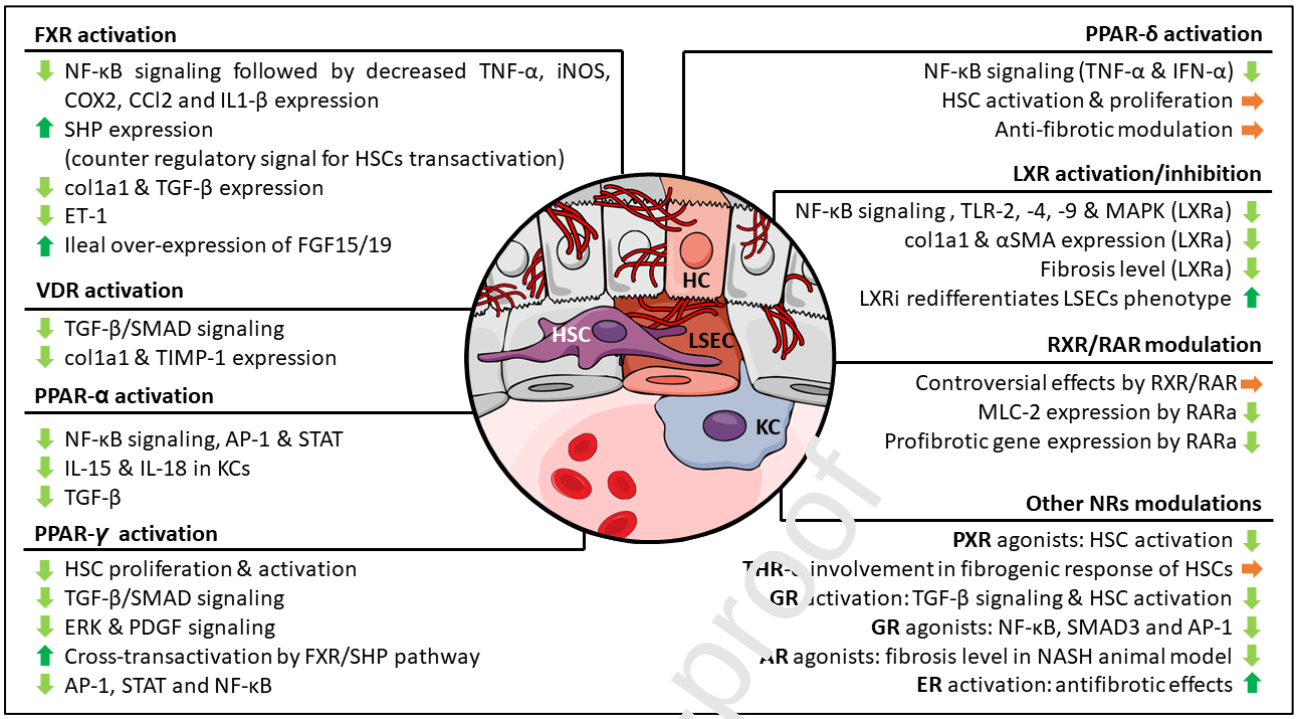

FIGURE 5. Molecular effects of NR modulators as potential treatment for liver fibrosis that are predominantly reported to occur within HSCs. Arrows indicate the presumed effects on liver fibrosis by upregulation/stimulation/activation (dark green) or downregulation/inhibition/blockade (light green) of the indicated signaling pathways. Orange arrows indicate effects of NR modulation on cell-specific responses or signaling pathways that are either controversial or yet insufficiently assessed.

TABLE 1. Reported effects of NR modulation, cellular expression pattern and discussion of controversial data.

FXR agonists	**NR expression pattern in liver fibrosis & contradictory effects of NR modulation**	**Discussion of controversial data and open research questions**
Reduction of liver fibrosis & HSC activation[58-60] Reduction of hepatic inflammation[27, 52, 53] Reduction of bile acid synthesis[49] Increased bile acid circulation[48] Increased cholesterol circulation[50] Increased triglyceride metabolism[50]	FXR activation and expression decreases during liver fibrosis.[48] • Primarily expressed in hepatocytes.[48, 57] • Marginally expressed in human HSCs.[48, 57] • Non-detectable expression in mouse HSCs[57] • FXR-related effects on gene and protein expression in an immortalized rat HSC cell line (HSC-T6) and primary cultures of rat HSCs.[61]	• Controversial data on FXR expression in HSCs. • Potential species- or strain-specific differences in FXR expression. • FXR-β is a functional NR only in rodents but appears only as pseudogene in the human genome.[46, 47]
VDR agonists		
Reduced fibrosis development[97, 98]	• Generally low expression of VDR in liver tissue.[93, 207] • Mainly expressed within hepatic	• VDR activation has likely limited efficacy as antifibrotic strategy but

	non-parenchymal cells, such as HSCs and BECs.[94, 208]	may help to inhibit fibrogenesis and promote fibrosis regression.
PPAR-α agonists		
Reduction of HSC activation Reduction of Inflammation[110] Increased fatty acid metabolism [209, 210] Fatty acid transport [209, 210] Increased peroxisome proliferation [210] Lowers hypertriglyceridemia [209, 210]	• Hepatic PPAR-α is more abundant than the PPAR subtypes -γ/-δ.[108] • Inter-species expression differences in PPAR-α are well documented but contradictory.[123] • PPAR-α is mainly expressed in hepatocytes.[108] • PPAR expression is generally reduced in human cirrhotic liver tissue and PPAR-α is downregulated in most liver cells during liver disease.[108] • PPAR-α is upregulated in LSECs during liver injury.[108]	• Further studies are needed to validate expression patterns of PPAR-α across different liver disease etiologies and stages. • Inter-species differences in PPAR-α expression in different liver cell types limits the comparability of preclinical studies.
PPAR-γ agonists		
Reduction of HSC activation Reduction of inflammation[212] Increased lipid storage[213]	• Hepatic PPAR-γ expression is generally reduced in human cirrhotic liver tissue[108] and loss of PPAR-γ expression and transcriptional activity is coupled with HSC activation.[129, 134] • PPAR-γ is mainly expressed in KCs,[108] highly expressed in qHSCs compared to hepatocytes and KCs,[211] seems to be upregulated in hepatocytes during liver injury (in this study also in HSCs)[108] but reduced expression of PPAR-γ in HSCs in BDL animal models[126]	• Contradictory data on up-/down-regulated expression of PPAR-γ in HSCs during liver injury. • Discrepancies about the role of PPAR-γ in fibrogenesis, next to high relevance in hepatic lipid metabolism.
PPAR-δ agonists		
Controversial effects in liver fibrosis Controversial effects on inflammation[214, 215] Altered fatty acid metabolism[216]	• Hepatic PPAR-δ expression is generally reduced in human cirrhotic liver tissue.[108] • PPAR-δ is mainly expression in LSECs and macrophages.[108] • PPAR-δ seems to be upregulated in LSECs/KCs during liver injury.[108] • PPAR-δ increased hepatoxicity by HSC activation & increased fibrosis markers[148]	• Controversial data on (cellular) PPAR-δ expression in liver fibrosis and controversial effects on liver fibrosis • Discrepancies in the hepatic cell specific expression pattern may relate to different severity levels and etiologies.
LXR modulators		
Controverse HSC activation[158] Reduced	• LXR expression correlated with the degree of hepatic steatosis,	• LXR agonists, inverse agonists, and the

inflammation[160-162] Reduced leucocyte recruitment[218] Increased cholesterol efflux[219] Increased lipogenesis[220] Increased gluconeogenesis[221]	inflammation and fibrosis.[217] • LXR-β is predominantly expressed in HSCs.[159] • increased LXR-α expression has been detected in qHSCs.[159] • LXR agonism promotes re-differentiation of primary LSECs.[165] • LXR inverse agonist SR9243 showed antifibrotic effects in NASH.[167] • Synthetic inhibitor SR928 showed improvement in liver fibrosis severity in a NASH model.[168]	inhibition of LXR activation all led to beneficial effects on liver fibrosis, resulting in a controversial discussion on direct/indirect antifibrotic effects by LXR modulation. • Side effects by LXR-α activation: e.g., hyperlipidemia and liver steatosis. • Further research on LXR-β agonism is warranted.
RXR/RAR agonists		
Dose dependent anti- vs. pro-fibrotic effects	• Liver cells and predominantly HSCs express three isoforms of RXR: -α, -β and -γ[170] • RXR-α is the dominant isoform in cultured HSCs[171] • RXR acts as heterodimer-partner for several other NRs	• Role of RXR activation in liver fibrosis is controversially discussed.[173] • The phenotype of HSCs might depend on the presence of natural or synthetic retinoids, which may explain divergent results of previous studies.[177]
THR agonists		
Controversial antifibrotic effects (indirect vs. direct) Increased hepatic cholesterol efflux [222-225] Increased bile acid flow[226, 227] Decreased hepatic fat [222-224]	• Decreased THR expression in liver tissue during liver disease (THR-α and THR-β).[192] • Several beneficial effects by THR-β activation reported in NAFLD and NASH • THR-α is predominant in HSCs and potentially harbors a crucial role in HSC cell differentiation.[192]	• Potential - but unlikely direct - antifibrotic effects by THR-β. • Side effects by non-liver THR-α activation: e.g., cardiotoxicity.[189] • Role of THR-α and THR-β in HSCs and in liver fibrosis needs further investigation.

TABLE-2: Clinical studies using pharmacological modulators of nuclear receptors and including assessment of fibrosis as primary or secondary endpoints. (Extracted from clinicaltrials.gov accessed on 04-July-2021). * Liver fibrosis-related readouts

Mechanism	Agent	Trial Number	Phase	Disease	(1) primary endpoint(s), (2) secondary endpoint(s)
THR-β agonist	VK2809	NCT04173065	2	NASH	(1) Liver fat by MRI-PDFF (2) NASH CRN score

					on liver biopsy*
THR-β agonist	Resmetirom (MGL-3196)	NCT04197479	3	NAFLD	(1) Adverse events (2) LDL-C, ApoB, Liver Fat by MRI-PDFF, TG, PRO-C3*
THR-β agonist	Resmetirom (MGL-3196)	NCT03900429	3	NASH (F2/F3)	(1) NASH resolution without worsening of fibrosis (NAS score on liver biopsy)* (2) All-cause mortality, progression to cirrhosis, liver-related clinical outcomes
PPAR-α agonist	Pemafibrate (K-877)	NCT03350165	2	NAFLD	(1) Liver fat by MRI-PDFF and safety (2) MRI-PDFF, MRE*, VCTE*; AST, ALT, GGT; CK18, HA*, collagen type IV*, M2BPGi, NAFLD fibrosis score*; FIB4*, NAFIC; ELF*
FXR agonist	Tropifexor (GS-9674)	NCT02854605	2	NASH	(1) Safety (TEAEs) (2) Noninvasive biomarkers of fibrosis* and steatosis
FXR agonist	EYP001a	NCT03812029	2	NASH	(1) Liver fat by MRI-PDFF and safety (2) Relative reduction in liver fat and liver iron content: ALT, AST, ProC3*, fibronectin, HA*
FXR agonist	OCA	NCT02548351 (REGENERATE)	3	NASH (F2/F3)	(1) Fibrosis stage improvement* without worsening of NASH or NASH resolution without worsening of liver fibrosis* (1) All cause death, MELD≥15; liver transplant; ascites; progression to histological cirrhosis; hospitalization for variceal bleeding, encephalopathy or SBP (2) ≥1 fibrosis stage improvement* AND/OR NASH

					resolution without worsening of either; no worsening of fibrosis* AND no worsening of NASH; ≥2 fibrosis stage improvement*; resolution of fibrosis*; histological progression to cirrhosis; liver biochemistry; biomarkers of liver function
FXR agonist	OCA	NCT03439254 (REVERSE)	3	NASH (F4)	(1) Effect on liver fibrosis improvement by histological NASH CRN score (2) ≥2 fibrosis stage improvement by histological Ishak score; NASH resolution by NASH CRN score
FXR agonist	OCA	NCT02308111 (COBALT)	4	PBC	(1) Death, liver transplant, MELD≥15, ascites; hospitalization for variceal bleeding, encephalopathy, or SBP (2) First occurrence of any event defining the primary endpoint; first occurrence of liver-related death; progression to cirrhosis; time to occurrence of HCC; bilirubin; AST, ALT, ALP, GGT, igM, TNFα, FGF19, CK18, ELF*, VCTE*
FXR agonist/ CCR2/5 antagonist	Tropifexor (LJN-452)/ Cenicriviroc	NCT03517540 (TANDEM)	2	NASH	(1) Safety by adverse events (2) ≥1 fibrosis stage improvement*, NASH resolution
FXR agonist/ dual SGLT1/2 inhibitor	Tropifexor (LJN-452)/ Licogliflozin	NCT04065841 (ELIVATE)	2	NASH	(1) Fibrosis improvement* without worsening of NASH; NASH resolution without worsening of

					fibrosis* (2) ≥fibrosis stage improvement*; 5% body weight reduction; liver fat content by MRI-PDFF; AST, AST, GGT; AEs and SAEs
GLP-1 receptor agonist/AAC inhibitor/ FXR agonist	Semaglutide/ Firsocostat/Cilofexor	NCT03987074	2	NASH	(1) Safety by AEs and SAEs (2) Fibrosis assessments*
AR agonist	Lipocine	NCT04134091	2	NASH	(1) Liver fat by MRI-PDFF (2) Change in NASH activity by histology; NAFLD resolution; NASH resolution; NASH resolution without worsening of liver fibrosis* by NASH CRN score; Change in liver fibrosis* by NASH SCRN score; change in HOMA-IR; AST, ALT, ALP, GGT, Bilirubin, CK; noninvasive fibrosis* and steatosis biomarkers; LDL, HDL, TG, change in biometric indices (body weight, BMI)
GR/MR modulator	Miricorilant (CORT118335)	NCT03823703	2	NASH	(1) Liver fat content by MRI-PDFF (2) Change in MRI-PDFF ≥30%; ProC3*; ELF*; AST; ALT

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Highlights:

1. Nuclear receptors (NRs) regulate key molecular signals of liver fibrogenesis
2. Agonists of FXR and PPAR isotypes have shown promising antifibrotic effects
3. Molecular effects of NR modulation are presented for different hepatic cells
4. The expression pattern of NRs varies across the spectrum of human liver disease
5. Clinical trials of NR modulators including fibrosis endpoints are summarized