Published in final edited form as:

J Exp Clin Med. 2009 December ; 1(1): 23–30. doi:10.1016/S1878-3317(09)60007-3.

Retinoic Acid-mediated Nuclear Receptor Activation and Hepatocyte Proliferation

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Abstract

Due to their well-known differentiation and apoptosis-inducing abilities, retinoic acid (RA) and its analogs have strong anti-cancer efficacy in human cancers. However, *in vivo* RA is a liver mitogen. While speculation has persisted that RA-mediated signaling is likely involved in hepatocyte proliferation during liver regeneration, direct evidence is still required. Findings in support of this proposition include observations that a release of retinyl palmitate (the precursor of RA) occurs in liver stellate cells following liver injury. Nevertheless, the biological action of this released vitamin A is virtually unknown. More likely is that the released vitamin A is converted to RA, the biological form, and then bound to a specific receptor (retinoid x receptor; RXR α), which is most abundantly expressed in the liver. Considering the mitogenic effects of RA, the RA-activated RXR α would likely then influence hepatocyte proliferation and liver tissue repair. At present, the mechanism by which RA stimulates hepatocyte proliferation is largely unknown. This review summarizes the activation of nuclear receptors (peroxisome proliferator activated receptor- α , pregnane x receptor, constitutive androstane receptor, and farnesoid x receptor) in an RXR α dependent manner to induce hepatocyte proliferation, providing a link between RA and its proliferative role.

Keywords

liver; nuclear receptor; proliferation; regeneration; retinoic acid

1. Retinoids

Vitamin A and its analogs, collectively termed retinoids, have a profound effect on cell growth, differentiation, apoptosis, and morphogenesis.^{1–3} While beta-carotene and retinoids at one time were considered promising chemopreventive or chemotherapeutic agents, a large-scale NIH population-based study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study) suggested that they increase lung cancer incidence.⁴ Since then, the potential benefits of retinoids in preventing or treating cancers have been a matter of controversy.

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Although efforts have been made to characterize the effects of retinoids on treating hepatoma and facilitating liver regeneration, the results have been ambiguous. In part, this is due to differences in the retinoids examined, their dosage, route of administration, and animal species.⁵⁻⁹ In contrast, retinoids conclusively stimulate hepatocyte proliferation with, for example, hypervitaminosis, a condition frequently associated with hepatomegaly.¹⁰ Administration of all-trans-retinoic acid (RA) induces hepatocyte proliferation in mice;¹¹ retinoids, including all-trans-RA, increase liver tumor incidence induced by diethylnitrosamine.^{12,13} In animals fed a vitamin A-deficient diet, hepatocyte proliferation is impaired.¹⁴ While the potential mitogenic effects of retinoids is of concern when considering their possible use in liver cancer treatment, these findings also imply the usefulness of retinoids in facilitating regeneration and tissue repair post-liver injury. To exploit these findings for clinical benefit, it is crucial to fully understand the mechanisms whereby retinoid-mediated signaling regulates liver cell proliferation.

More than 90% of total body retinol (retinyl palmitate) is stored in liver stellate cells.¹⁵ During hepatic injury, stellate cells are activated, with their transdifferentiation into fibroblasts associated with vitamin A depletion.^{16,17} Surprisingly, the biological effect of released vitamin A during liver injury is unknown. A reasonable hypothesis is that the released vitamin A is converted to RA, the biologically active form. Inasmuch as RA has a mitogenic effect, RA-mediated signaling pathways might play a role in tissue repair during liver injury. The purpose of this review is to summarize the mechanism by which RA can activate nuclear receptors and stimulate hepatocyte proliferation, perhaps helping to facilitate tissue repair.

2. Nuclear Receptors—Structure and Classification

The action of RA is mediated through nuclear receptors, the transcription factors that regulate diverse processes including reproduction, embryonic development, cell differentiation, and cellular homeostasis.¹⁸⁻²² They are modular in structure and contain the following domains: (1) N-terminal regulatory domain, which contains activation function 1 (AF1) of whose action is independent in the presence of ligand. To regulate gene transcription, AF1 synergizes with AF2, which is located within the ligand binding domain. (2) DNA binding domain (DBD) is a highly conserved domain featuring two zinc fingers that bind to a specific DNA sequence named the hormone response element. (3) Hinge domain is a flexible domain that regulates intracellular localization of the nuclear receptor. (4) Ligand binding domain contains an alpha helical sandwich folding that allows ligand binding. The ligand binding domain also contains a dimerization domain that binds to coregulators. In addition, the action of AF2, located within the ligand binding domain, is ligand dependent. (5) The C-terminal domain contains a variable sequence between nuclear receptors.^{18,23} Different nuclear receptors bind to their response elements as homodimers, as heterodimers with retinoid x receptors (RXRs), or as monomers. Binding of ligand to the ligand binding domain elicits a series of sequential reactions including the conformational changes in the receptors, release of the co-repressor complex, and recruitment of coactivators.^{23,24} Consequently, the activated nuclear receptor induces target gene expression. Comprised of at least 49 members, nuclear receptors are expressed differentially among tissues, some in a circadian manner.²⁵ Nevertheless, there are exceptions, for

example, small heterodimer partner (SHP) lacks a DBD and yet heterodimerizes with other nuclear receptors, often in a ligand dependent manner.^{26–28}

The nuclear receptor family is classified into three groups:¹⁸ steroid hormone receptors, adopted orphan nuclear receptors, and orphan nuclear receptors. (1) Steroid hormone receptors generally bind DNA as homodimers and are activated with high affinity by steroid hormones. Members of this group include the glucocorticoids (GR), mineralocorticoids (MR), estrogen (ER), androgen (AR), and progesterone receptors (PR). (2) The adopted orphan nuclear receptors function as heterodimers with RXRs. As the ligands for the specific receptors are identified, these orphan receptors are considered adopted. Members of this group include the receptors for fatty acids (peroxisome proliferator activated receptors, PPARs), bile acids (farnesoid x receptor, FXR), oxysterols (liver x receptor, LXR), xenobiotics (pregnane x receptor, PXR; constitutive androstane receptor, CAR), and RAs (RA receptor, RAR). (3) The orphan nuclear receptors refer to transcription factors, which share a common sequence with other nuclear receptors. At present, no specific ligands have been identified or appear to lack a functional ligand binding domain based on structural analysis. Members of this group include nerve growth factor-induced clone B (Nur77), nuclear receptor related-1 (Nurr1), neuron-derived orphan receptor-1 (NOR1), SHP, liver related homologue-1 (LRH-1), hepatocyte nuclear factor 4 α (HNF4 α), and so on.¹⁸

3. Nuclear Receptors and Hepatocyte Proliferation

The receptors for RA include RAR (α , β , and γ) and RXR (α , β , and γ). RARs can be activated by all-trans RA and 9-cis RA, but RXRs are exclusively activated by 9-cis RA.^{29,30} At high concentrations, all-trans RA converts into 9-cis RA and can activate RXRs. As mentioned above, the RXR is unique in this superfamily in that it forms both homodimers and heterodimerizes with others that include PPARs, CAR, PXR, as well as others.^{23,31} RXR can be either an active (permissive) or silent (non-permissive) partner.²³ When acting as a silent partner, RA cannot activate the heterodimer. Conversely, when an active partner, both RA and the ligand for the partner can activate the heterodimer. For example, as RXR α is an active partner of PPAR α , the RXR α /PPAR α can be activated by 9-cis RA and PPAR α agonist hypolipidemic drugs like Wy14,643; the addition of both has a synergistic effect on the activation of RXR α /PPAR α . Besides PPAR α , RA can also activate RXR α /PXR³² and RXR α /CAR,³³ and therefore RXR α is the permissive partner of PXR and CAR.

Among RA receptors, RXR α is most abundantly expressed in the liver; the liver has the highest level of RXR α of all the organs. To understand the biological effect of RA and its receptor in the liver, we have generated hepatocyte RXR α -deficient mice.³⁴ Using this model, the essential role of hepatocyte RXR α in lipid,³⁵ carbohydrate,³⁶ xenobiotic,^{37–39} and amino acid⁴⁰ metabolism and homeostasis has been illustrated.

In the process of hepatocyte proliferation, many nuclear receptor-mediated signals are implicated. For example, the activation of PPAR α , CAR, PXR, and FXR causes hepatomegaly or liver cancer.^{41–44} Partial hepatectomy-induced liver regeneration is impaired in mice lacking these nuclear receptors. Since RA can activate nuclear receptor-mediated pathways, it is likely that mediation via PPAR α , PXR, CAR, and FXR, RA can

exert its mitogenic effect in the liver. The following summarizes the actions of these four nuclear receptors in regulating hepatocyte proliferation.

3.1. PPAR α

PPAR α derives its name from its ability to be activated by peroxisome proliferator chemicals.⁴⁵ Studies using PPAR α ligands⁴⁶ and PPAR α -null mice⁴⁷ have elucidated the role of PPAR α in lipid homeostasis. Starvation activates PPAR α and increases fatty acid β -oxidation.⁴⁸ The natural ligands for PPAR α , which are thought to activate PPAR α during starvation, are likely saturated and unsaturated fatty acids. Synthetic PPAR α ligands, including the widely prescribed lipid-lowering fibrates such as clofibrate and fenofibrate, are used to reduce plasma triglycerides. Our studies have shown that activation of PPAR α by Wy14,643 reduces hepatic triglyceride levels, and that this effect is blunted during hepatocyte RXR α deficiencies. This indicates that the hypolipidemic effect of PPAR α is dependent on the presence of hepatocyte RXR α .^{44,49}

Treating rodents with Wy14,643 results in an increased incidence of liver tumors through a PPAR α -dependent mechanism,⁴³ although the mechanism of action for this PPAR α -mediated effect is largely unknown. Our work⁵⁰ and those of others²⁵ have established that PPARs (α , β , and γ) are circadian genes. Among our studies is expression profiling of the diurnal hepatic genes encoding circadian clocks, nuclear receptors, and lipid metabolic enzymes.⁵⁰ The Cosinor analysis revealed that several genes encoding nuclear receptors, including PPARs and lipid metabolizing enzymes, and genes whose expressions are regulated by PPARs, are rhythmically expressed in phase with peripheral clocks. These correlate with diurnal changes of hepatic lipid levels. The activation of PPAR α regulates the expression of clock genes, which in turn control cell cycle progression. Activation of PPAR α induces the expression of brain and muscle Arnt-like protein 1 (Bmal1) and its downstream Rev-erba, which in turn negatively regulates cyclin-dependent kinase inhibitor 1A (P21) and thus facilitates G1/S transition.⁵¹ The treatment of mice with PPAR α activator induces cyclin D1 and D3 protein levels; activation of PPAR α also inhibits the expression of Wee1 kinase and keeps the CDC2/cyclin B1 complex from inactivation. Moreover, activation of PPAR α increases the level of CDC2 mRNA in mouse livers. Thus, PPAR α activation not only activates CDC2 by preventing Wee1-mediated CDC2 phosphorylation, but also induces the transcription of the CDC2 gene, leading to G2/M transition.⁴⁴ In addition, activation of PPAR α -mediated cell cycle gene expressions were either not found or greatly reduced due to hepatocyte RXR α deficiency. These findings suggest the dual role of PPAR α in regulating the cell cycle; PPAR α regulates G1/S transition as well as Wee1/CDC2/cyclin B1-mediated G2/M transition. Such an effect is hepatocyte RXR α dependent.⁴⁴ The action of PPAR α in regulating circadian cell cycle genes is summarized in Figure 1.

In comparison with other nuclear receptors, PPAR α is unique in that it has species specificity. The activation of rodent PPAR α induces hepatomegaly, although in humans such an effect is not observed. It has been shown that the species response difference to the cancer-producing effect of Wy14,643 is due to the differential expression of microRNA Let-7c, which controls the levels of c-Myc by destabilizing its mRNA.⁵² In rodents, the

hepatomegaly effect of the PPAR α activator is due to the inhibition of Let-7c, whereas Wy14,643-mediated inhibition of Let-7c is not found in humanized PPAR α mice and thus results in no hepatomegaly.⁵² Despite the species difference of PPAR α in responding to PPAR α activators, it is unlikely that there is a species difference in response to endogenous ligands. Fatty acids and RAs can be the potential endogenous ligands (activators) for PPAR α . Accordingly, the question of whether human PPAR α can regulate circadian cell cycle progression requires further study.

Several studies that have examined the role of PPAR α in liver regeneration have been controversial.^{53–56} This controversy may be due to differences in the time points studied, in the parameters used to evaluate proliferation, or in the genetic backgrounds of the mice. Two of the studies featured negative findings, indicating that no impairment was observed in regenerating PPAR α -null livers.^{55,56} Another two studies demonstrated impairment of liver regeneration.

Anderson's group studied liver regeneration in PPAR α knockout mice 1, 2, 3, and 7 days post-partial hepatectomy.⁵⁴ PPAR α knockout mice had a 12–24-hour delay in liver regeneration associated with a delayed onset of DNA synthesis, which is consistent with our findings generated using hepatocyte RXR α -deficient mice.⁵⁷ In addition, delayed hepatic expression of cyclin D1 and c-Myc was also reported. The conclusion was that delayed cell cycle gene expression and altered lipid homeostasis were mainly due to inactivation of Cyp4a14, contributing to the impaired regeneration in PPAR α knockout mice. The mechanism by which cyclin D1 gene expression was delayed in regenerating PPAR α -null liver was not studied.

Wheeler's group studied the role of PPAR α -dependent isoprenoid synthesis in liver regeneration within 72 hours after partial hepatectomy.⁵³ DNA synthesis was inhibited at 42 and 48 hours post-partial hepatectomy in PPAR α -null mice; however, no significant difference was observed at 72 hours post-partial hepatectomy. The induction of key enzymes involved in isoprenoid synthesis was suppressed in PPAR α -null mice. Accordingly, prenylation and the activation of small GTPases including Ras and RhoA, which subsequently activate cyclin-dependent kinase, were impaired due to PPAR α deficiency. Their conclusion was that PPAR α -mediated isoprenoid synthesis and subsequent activation of Ras and RhoA play a key role in the regulation of cell cycle and cell proliferation.⁵³ In light of positive findings, different mechanisms were proposed. Thus, the results are conflicting and the role of PPAR α in liver regeneration remains unclear.

3.2. PXR

Nuclear receptor pregnane X receptor (PXR, NR1I2) regulates the expression of a range of genes associated with endobiotic and xenobiotic clearance^{58–60} and is known as a sensor for xenobiotic biotransformation. Although mouse and human PXR are products of a single divergent gene,^{61,62} they are pharmacologically distinct because their activators seldom overlap. Thus, substances that typically activate human PXR usually have little effect on the mouse form of this enzyme. For example, rifampin, an antituberculosis agent also known as rifampicin, phytoestrogens, and corticosterone, potently activates human PXR, but not mouse PXR. In contrast, pregnenolone-16 α -carbonitrile (PCN, EC₅₀ = 10⁻⁶ M) and

dexamethasone strongly enhance mouse, though not human, PXR.⁶³ Despite their selectivities, both human and mouse PXR have the same DNA-binding specificity and regulate expression of CYP3A genes.

PXR is also implicated in hepatocyte proliferation and liver regeneration. The activation of PXR by PCN causes hepatomegaly.⁶⁴ Our published data indicate that PXR mediates hepatocyte proliferation during both the early and late stages of liver regeneration, with a lack of PXR impairing the normal progression of this recovery process.⁶⁵ PXR activation causes decreased proliferation in myofibroblasts,⁶⁶ perhaps offering a way for the liver to protect against liver fibrosis. Clearly, the mechanisms wherein PXR regulates proliferation require further investigation. Besides proliferative and antifibrosis, PXR also has an anti-inflammatory mechanism. Although inflammation and fibrosis are not the focus of the current review, these findings suggest another important hepatoprotective role of PXR.⁶⁷ Other groups as well as our own have shown that RA³² and β -carotene⁶⁸ can activate the PXR/RXR α -mediated pathway, implying that retinoids can mediate hepatocyte proliferation via PXR activation. Due to this, retinoids might also be able to facilitate xenobiotic metabolism and potentially cause drug-drug interaction or drug resistance.

3.3. CAR

The nuclear receptor constitutive androstane receptor (CAR, NR1I3) regulates hepatic genes involved in xenobiotic detoxification as well as genes involved in energy homeostasis. CAR is a central regulator of xenobiotic metabolism. Its activation by phenobarbital-related compounds induces hepatic expression of detoxification enzymes and transporters as well as increased liver size. CAR-mediated hepatomegaly is a transient, adaptive response to acute xenobiotic stress. In contrast, chronic CAR activation results in hepatocarcinogenesis.⁴² In both acute and chronic xenobiotic responses, hepatocyte DNA replication is increased and apoptosis decreased in a CAR-dependent manner. These results demonstrate the essential role of CAR in regulating both liver homeostasis and tumorigenesis in response to xenobiotic stresses. TCPOBOP (1,4-bis-[2-(3,5-dichloropyridyloxy)] benzene, 3,3',5,5'-tetrachloro-1,4-bis(pyridyloxy)benzene), a CAR agonist, has been shown to cause similar growth in mouse livers. C-Myc and its target FoxM1 are critical downstream effectors of TCPOBOP/CAR-mediated direct liver hyperplasia.⁶⁹ These findings strengthen the notion that CAR can directly affect cellular proliferation. A transient delay in hepatocyte proliferation is also found in CAR knockout mice.⁷⁰ Moreover, evidence indicates the presence of cross-talk between PXR/CAR and PPAR α . The expression of PPAR α and its downstream target genes are activated in PXR/CAR double knockout mice. Phenobarbital can activate the transcription of PPAR α via direct binding to the HNF4 α response element.⁷¹ Our recent publication indicated the capability of RA in activating CAR, which provides another potential pathway for RA to exert its mitogenic effect.³³

3.4. FXR

The farnesoid x receptor (FXR), also known as NR1H4, is expressed in tissues exposed to high concentrations of bile acids, such as the liver, intestine, and kidney. Chenodeoxycholic acid (CDCA) and other bile acids are natural ligands for FXR, which plays an essential role

in maintaining bile acid, cholesterol, and glucose homeostasis.⁷² The hydrophobic bile acid CDCA is the most effective FXR activator.

An intriguing link between FXR and hepatocarcinogenesis has been identified. FXR knockout mice display prominent liver injury and inflammation from 9 to 12 months of age. When the FXR knockout mice are 15 months old, hepatocellular adenoma and carcinoma (but not other types of tumors), spontaneously develop with elevated expressions of inflammatory genes (INF- γ , TNF- α , and IL-6). In contrast, no liver tumors are observed in wild-type mice of the same age.⁷³ Diet enriched with bile acid strongly promotes N-nitrosodiethylamine-initiated liver tumorigenesis, whereas lowering the bile acid pool in FXR knockout mice significantly reduces malignant lesions. These results suggest that increased expression of proinflammatory genes in aging FXR knockout mice may eventually contribute to liver carcinogenesis.⁷³ FXR-deficient mice display a transient delay in partial hepatectomy-induced liver regeneration.⁷⁰ Since RXR α is a permissive partner of FXR, the potential effect of RA in regulating bile acid homeostasis and hepatocyte proliferation requires investigation.

4. Cellular Binding Proteins Dictate the Effect of RA

Cytosolic transport and stabilization of hydrophobic retinoids is made possible by retinoid binding proteins. These proteins are also important for mediating the biological activity of retinoids.^{74,75} Both cellular RA binding proteins (CRABP) I and II display a high affinity for RA. CRABP I decreases the cellular responses to RA by promoting its degradation, whereas CRABP II does so by delivering RA to RAR, facilitating RAR-mediated differentiation.^{74,76} CRABP I and II belong to a binding protein family that includes fatty-acid binding proteins (FABPs).⁷⁷ Keratinocyte FABP (FABP 5) and adipocyte FABP (FABP 4) respectively enhance the transcriptional activity of PPAR β and PPAR γ .⁷⁵ RA selectively activates PPAR β with nanomolar affinity,⁷⁸ modulating the conformation of PPAR β , promoting its interaction with the coactivator SRC-1, and activating PPAR β -mediated transcription.⁷⁸ The cellular expression ratio of FABP5/CRABP II determines which nuclear receptor is activated by RA. Thus, RA activates RAR in MCF-7 mammary carcinoma cells, which have a low FABP5/CRABP II ratio. Conversely, RA activates PPAR β in keratinocytes, which have a high ratio of FABP5/CRABP II. Decreasing this ratio in keratinocytes, either through overexpressing CRABP II or knocking down FABP5, converts RA from a prosurvival to a proapoptotic factor.⁷⁹ Thus, these binding proteins appear to channel ligands to a particular receptor and, in that way, regulate a select pathway. Data suggest that the opposing roles of RA in cell proliferation and apoptosis might be binding protein specific.

5. Summary

The liver is unique among major organs in its extraordinary ability to regenerate following injury. Liver regeneration is an orchestrated series of biological processes that includes sequential changes in gene expression, growth factor production, and tissue remodeling. While hepatic stem cells are not significantly involved in liver regeneration, hepatocytes, which are not terminally differentiated, display substantial proliferative capacity.^{80–83} Partial

hepatectomy is the most common procedure used for studying hepatocyte proliferation and liver regeneration. Following partial hepatectomy, hepatocytes move from G0 to G1, traverse to DNA synthesis or S phase, and ultimately undergo mitosis. Factors known to participate in partial hepatectomy-induced liver regeneration are classified into five categories.^{80–83} Included are priming factors (IL-6 and TNF- α), which render hepatocytes competent to respond to growth factors. Growth factors (HGF, TGF- α , and EGF) allow competent hepatocytes to progress through the cell cycle from G1 to S phase, whereas co-mitogens (insulin and epinephrine) facilitate mitogen activity. A fourth category are the tonic growth inhibitors (activin A and TGF- β), which suppress the growth response, and the fifth are suppressors of growth inhibitors (follistatin), which inhibit the activity of tonic growth inhibitors. The absence of any one of these factors does not permanently impair liver regeneration.^{80–83} Although HGF is the most potent hepatic mitogen *in vitro*, it produces minimal liver cell proliferation in normal animals.⁸⁴ While IL-6 is a potent liver mitogen,⁸³ it cannot be used to stimulate human liver regeneration because of serious side effects. Moreover, the growth-inducing property of TNF- α and IL-6 *in vitro* remains a matter of controversy. Given these problems, study of the impact of retinoids in liver regeneration and tissue repair merits consideration.

While RAs are well known to cause differentiation and display apoptotic effects, they can also increase liver cancer incidence. RAs, whose precursor retinyl palmitate is stored primarily in the liver, are known liver mitogens. Surprisingly, the mechanism of the mitogenic effect of RAs is unknown. Because RAs are only mild mitogens, they are approved by the FDA for human use. However, this mild mitogenic effect should not be ignored because agents are routinely used to treat psoriasis, acne, and cancer. It is important, therefore, to understand in detail the diverse effects of RA in cell differentiation and proliferation as this would make it possible to maximize their use in chemoprevention and chemotherapy. In summary, existing data indicate that RA can activate PPAR α , PXR, CAR, and FXR pathways, which could potentially mediate its mitogenic effect (illustrated in Figure 2). Additional experimentation is required to define this system further. Such knowledge may direct the development of novel therapeutics that facilitate liver tissue repair or terminate deregulated liver cell proliferation.

Acknowledgments

This work is supported by NIH grants CA 53596 and AA14147.

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Figure 1.

Peroxisome proliferator activated receptor- α (PPAR α) has a dual role in regulating the cell cycle; activation of PPAR α stimulates G1/S as well as G2/M transition. Activation of PPAR α induces the expression of brain and muscle Arnt-like protein 1 (Bmal1) and its downstream Rev-erb α , which negatively regulates cyclin-dependent kinase (CDK) inhibitor 1A (P21) and thus facilitates G1/S transition. Activation of PPAR α also inhibits the expression of Wee1 kinase and keeps the CDC2/cyclin B complex from inactivation. In addition, activation of PPAR α increases the level of CDC2 mRNA in mouse liver. Thus, PPAR α activation not only activates CDC2 by preventing Wee1-mediated CDC2 phosphorylation, but also induces the transcription of the CDC2 gene, thus leading to G2/M transition.

Figure 2.

The proposed mechanisms by which retinoic acids can induce hepatocyte proliferation. By binding to hepatic retinoid x receptor (RXR α), retinoic acids can activate peroxisome proliferator activated receptor- α (PPAR α), pregnane x receptor (PXR), constitutive androstane receptor (CAR), farnesoid x receptor (FXR) and their downstream pathways to regulate hepatocyte proliferation. In addition, nuclear receptors cross talk and have redundant effects.