MINI REVIEW

Role of nuclear receptors in hepatitis B and C infections

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Summary

Nuclear receptors are key regulators of many cellular functions including energy supply by the direct control of the expression of target genes. They constitute a super-family of transcription factors activated by ligands, hormones or metabolites, and therefore, sensible to host metabolic stimuli. Viral replication and production requires energy and elementary building blocks from the infected cells. Hepatitis B and C virus replication is modulated in part by liver nuclear receptors that regulate the glucose and lipid metabolism. However, nuclear receptors control the two viruses’ replication by different mechanisms. The expression of hepatitis B virus genes is directly under the control of nuclear receptors, which bind to the viral genome regulatory regions. Viral replication and production may, therefore, be optimal when cells receive the correct metabolic signals. Hepatitis C virus replication and production depend to a large extent on lipidogenesis and lipoprotein secretion. The role of nuclear receptors in controlling hepatitis C replication may be to turn on the cellular mode that would provide the appropriate metabolic environment for viral replication.

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Introduction

Viruses depend on the cell they infect for their replication. In particular, infected cells must provide energy and essential building blocks for supporting viral replication and assembly. The ability of cells to respond to these viral requirements by shifting their metabolism contributes to the tissue tropism of viruses. The liver is a highly specialized tissue, which regulates the host metabolism in response to exogenous situations such as feeding and fasting. This regulation is achieved by a combination of ubiquitous and liver specific nuclear factors and receptors whose activity is naturally modulated by the host conditions. Hepatitis B and C viruses (HBV and HCV) whose tropism is restricted to the liver have recently been shown to perturb the activity of these factors and to take advantage of certain metabolic stimuli.

Nuclear receptors (NRs) belong to a family of ligand-dependant transcription factors that play a key role in
cell differentiation and metabolism [1,2]. Ligands of NRs include hormones, vitamins and a variety of metabolic products. Ligand binding on NRs induces a dramatic conformational change, which leads to the release of corepressors and the recruitment of coactivators [3]. These cofactors influence NR-mediated transcription through modulation of the chromatin structure and stabilization of the transcription machinery [4]. NR expression is either ubiquitous or restricted to some organs where they play a crucial role in the control of specific functions. The metabolic functions of the liver are predominantly regulated by specific nuclear factors and receptors. This review focuses on the role of liver NRs in the control of HBV and HCV replication within the complex interplay of nuclear factors that regulate the metabolic status of the host.

Hepatitis B

HBV infection is limited to hepatocytes of humans and primates. Despite an efficient vaccine, HBV infection remains a major public health problem with more than 400 millions chronically infected people worldwide, who are at high risk of developing cirrhosis and hepatocellular carcinoma [5].

HBV expression and replication cycle

HBV is an enveloped virus containing a 3.2-kb partially double-stranded DNA genome, which consist of a full-length negative strand and a shorter positive strand [6,7]. After virions entry into hepatocytes by an unknown receptor [8], nucleocapsids transport the genomic HBV DNA to the nucleus where the relaxed circular DNA is converted to covalently closed circular DNA (cccDNA). The cccDNA persists in the nucleus as a mini-chromosome, which contains four open reading frames. These open reading frames encode the reverse transcriptase, precore, and core proteins; three surface antigen proteins (Long, Medium and Short HBsAg); and the X protein. Regulation of HBV transcription is under the control of four promoters (the core, pre-S1, pre-S2/S, and X promoters) and two enhancer promoters (the EN1 and the S and X promoters) whereas the EN2-core promoter region is recognized mainly by hepatic NRs, which are only active in liver cells. Within this later region (Fig. 1), one NR response element (NRRE) at position 1757 is competitively recognized by six NRs, namely hepatocyte nuclear factor 4 alpha (HNF4), RXR-peroxisome proliferator-activated receptor alpha (PPARα) heterodimers, RXR-farnesoid X receptor (FXR) heterodimers, liver receptor homolog-1 (LRH-1), estrogen-related receptors (ERR), chicken ovalbumin upstream promoter transcription factor (COUP-TF) and the testicular orphan receptor 2 and 4 (TR2 and TR4). Another NRRE (at position 1635) is recognized by TR2 and 4, LRH-1 and ERR while three other NRREs, scattered between the two above mentioned NRRE, are distinctive binding sites for HNF4, FXR-RXR or LRH-1 [11—15]. In hepatoma cell lines, binding of HNF4, PPAR/RXR, FXR/RXR, LRH1 and the ERRs to their respective NRREs increases synthesis of the pregenomic RNA and of viral replication intermediates whereas COUP-TF1, TR2 and TR4 exert an inhibitory effect on viral replication [12,16,17]. Interestingly, in non-hepatoma cell lines that do not support HBV replication, exogenous expression of only one of these NRs (except COUP-TF1, TR2 and TR4) is sufficient to initiate the synthesis of the pregenomic RNA and replication intermediates [15]. These observations indicate that these liver-enriched NRs controlling the EN2-core promoter region are critical for HBV replication and that they participate to the hepatic tropism of the virus. However, the specific role of each NR may be difficult to assess since they can bind to identical NRREs (Fig. 1). The scheme gets even another degree of complexity with the mutual regulatory interactions that modulate the expression of the NRs. As examples, HNF4α regulates the expression of LRH1, PPARα and FXR; LRH1 activates HNF4 transcription and finally, PPARα reciprocally modulates ERRα and FXR synthesis [18—20].

The HBV mini-chromosome

Although informative, these in vitro experiments using reporter genes under the control of the EN2-core promoter region or greater-than-one HBV genome construct do not reflect the complexity of the control of HBV genome expression in vivo. HBV cccDNA accumulates in the nucleus of infected hepatocytes as a stable mini-chromosome with a chromatin-like structure composed of up to 20 nucleosomes organized by histones, cellular transcription factors and chromatin modifying enzymes as well as core and HBx viral proteins [21,22]. In general, the accessibility of transcription factors and NRs to their DNA target sequences is regulated by non-DNA binding coactivators or histone deacetylases (HDAC) that modify the histone acetylation status. Existence in the liver of less compacted HBV genome suggests that epigenetic control of histone acetylation is an important factor that regulates the accessibility of NRs to viral DNA and, consequently, modulates viral transcription and replication [23]. Immunoprecipitation of cccDNA-specific chromatin (ChIP) shows that recruitment of histone acetyl transferases (p300/CBP, PCAF, GCN5) proportionally induces viral transcription and replication while histone deacetylase 1 (HDAC) recruitment on cccDNA reduces HBV RNA synthesis [24].
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Control of NR activity on HBV genome transcription

Factors known to be involved in the epigenetic control of HBV RNA transcription include the viral protein HBx, which favors histone acetylation by the recruitment of p300 on the HBV mini-chromosome [25]. Several cellular cofactors also cooperate with the NRs to modulate transcription of the HBV genes. For instance, the acetylase p300 and the deacetylase SIRT1, also identified by ChIP on the HBV minichromosome, modify the activity of FXR through modulation of its acetylation [26]. The PPAR-coactivator 1 alpha (PGC-1) is a co-activator of PPARγ but also of many NRs including the PPARs, HNF4, ERRs and FXR and of transcription factors as FOXO1 [27]. Accordingly, PGC-1 can co-activate nuclear-dependent HBV genome expression. Conversely, the small heterodimer partner (SHP) co-repressor, which is a FXR target gene, down regulates the HNF4 dependant HBV expression and represses the positive effect of PGC-1 on all NRs [14]. It becomes thus difficult to distinguish what NRs and transcription factors principally regulate the transcription of the HBV RNAs in various metabolic conditions that differently activate them. The global effects of metabolic stimuli have been evaluated in mouse models of HBV infection. Micro-injection of HBV DNA in the tail induces transient HBV expression in the liver, which is dependant on the metabolic status of the mice [28]. A short-term fasting stimulated strongly the gluconeogenic program and induced HBV gene expression HBV in a PGC-1 dependant manner while refeeding reversed this induction. However, another study reported only a limited effect of fasting on HBV expression in a transgenic mouse model of HBV infection [29].

Hepatitis C

Hepatitis C is not only a major public health problem with 170 millions people infected worldwide at risk of cirrhosis and hepatocellular carcinoma but also a puzzling disease, tightly associated with host metabolic modifications. In chronically-infected patients, these modifications consist in a specific metabolic syndrome including insulin resistance (IR) and liver steatosis [30,31]. This link between hepatitis C and metabolism is further strengthened by the association of the virus with triacylglycerol (TAG) rich lipoproteins (TRL), i.e. very low, intermediate and low-density lipoproteins (VLDL, IDL and LDL) [32]. These hybrid lipoprotein-virus particles, distinctive of HCV, are highly infectious and were designated lipo-viral-particles (LVP) [33].

HCV induced metabolism modifications

IR and type 2 diabetes mellitus are more prevalent in chronic hepatitis C (CHC) than in any other liver diseases or in age control matched general population [34—38]. A common metabolic consequence of IR is the mobilization of fatty acids from adipose tissue and their uptake by the liver with the possible induction of fatty liver. Accordingly, IR is a variable independently related to fatty liver in CHC [32]. Steatosis defined as an increased fat content of the liver, essentially TAG, is a very common feature of CHC. If it is observed for all genotypes, marked steatosis defined as more than 20% of hepatocytes containing micro/macroversicular changes is more closely related to genotype 3-CHC (CHC-3) than to CHC-1 and -4 [39].
Figure 2  Major host pathways of the central carbon metabolism modified by HCV. 1. Insulin resistance may contribute to the mobilization of fatty acids from peripheral tissue and favor their uptake by hepatocytes. 2. Expression of lipogenic enzymes (citrate synthase, ATP citrate lyase, fatty acid synthase, FAS) is increased mainly through activation of nuclear receptors and transcription factors. 3. Translocation of fatty acyl-CoA from the cytosol into the mitochondrion may be impaired by down regulation of the expression of the carnitine palmitoyl acyl-CoA transferase 1 (CPTIA) resulting from the HCV induced PPARα activity inhibition. 4. The activity of MTP and apoB, the major proteins that control the secretion of VLDL with their cargo of neutral lipids, TAG and cholesterol esters, is inhibited by HCV core and NS5A proteins respectively. An increased accumulation of lipids in hepatocytes may result from these modifications.

One major unanswered question is whether accumulation of lipids within the cells is a prerequisite to viral replication and secretion of virions or just collateral effect of the infection. On one hand, excess of lipids stored in cytosolic lipid droplets have been shown to play a major role in the formation of infectious particles [40,41]. Lipid secretion by the liver may also be mandatory for the assembly and secretion of the VLDL-associated LVP. On the other hand, the high degree of association between steatosis and HCV replication for genotype 3, compared to other genotypes, suggests that the dependence on steatosis is not an absolute condition for viral replication [30]. Genome wide as well as –omic studies improved our understanding of the overall dynamic modifications of the metabolism induced by HCV with some insights on the consequences for viral replication [42—47]. Even if no data are today available on the fluxes of metabolites within the central carbon metabolism, infection seems to induce an active and rapid lipid synthesis and turnover characterized by the accumulation of some phospholipid classes while TAG abundance tends to decrease during the course of infection with the concomitant secretion of lipoprotein-associated virions [44]. Therefore, it was suggested that the viral ability to mobilize the lipids, but not that of inducing accumulation of lipids, drives the efficiency to produce infectious virions [48]. An intriguing hypothesis is then that HCV genotype 3 may mobilize lipids from the cytosolic lipid droplets less efficiently than the other genotypes with, therefore, a higher tendency to induce macroscopic steatosis.

Mechanisms of virus-induced steatosis and role of nuclear factors in fat accumulation

Fig. 2 summarizes the main pathways that can contribute to steatosis. As mentioned above, IR mobilizes fatty acids from peripheral tissue to the liver. Lipid secretion by the liver may also be impaired, contributing to accumulation of lipids in hepatocytes [48—50]. In a transgenic mouse model, HCV core protein was shown to inhibit the activity of the microsomal triglyceride transfer protein (MTP), which is essential for lipoprotein assembly and secretion [49]. Using the HCV subgenomic replicon system, it was shown that secretion of apoB100 and TAG is significantly reduced by the viral replication. Interaction between the HCV non structural protein 5A (NS5A) and apoB100 was suggested to favor the degradation of apoB [50].

Sterol regulatory element binding proteins (SREBPs) reside in the ER membrane from which they are released by cleavage when cellular cholesterol level decreases. Released SREBP N-terminal fragment is a transcription factor that translocates to the nucleus, binds to the sterol regulatory element in the promoter of lipidogenic genes to activate their expression. HCV core and NS4B of genotype 3 promote endoplasmic reticulum oxidative stress, which also induces the cleavage of SREBPs and activation of the lipidogenic genes transcription [51]. HCV core protein, particularly from genotype 3, transactivates the fatty acid synthase (FAS) promoter in an SREBP-1, PPARγ and RXR-dependant manner [52—54]. Among the NRs which regulate the lipid metabolism and whose functions could be modulated by CHC, PPARα is of primary importance but with apparent conflicting reports. First of all, expression of PPARα mRNA is down-regulated in the liver of infected patients and chimpanzees [47,55]. However, in 293T and CHO cell lines, the core protein can bind to RXR and enhance the transcriptional activity regulated by RXR homodimer and PPARα/RXR heterodimer [54]. The PPARα key target gene, carnitine palmitoyl acyl-CoA transferase 1 (CPTIA), expression is also down-regulated in patient liver [55]. CPTIA controls the translocation of fatty
acyl-CoA from the cytosol into the mitochondrion for β-oxidation. Inhibition of its expression during infection could, thus, contribute to the accumulation of fatty acids in the cytosol of infected hepatocytes. Interestingly, CPTIA mRNA is expressed at similar levels in HepG2 cells expressing or not HCV core, however, in core expressing cells, the increase of CPTIA expression after activation of PPARα by fibrates was prevented [55]. If the expression of PPARα is modulated during HCV infection, modulation of PPARα activity regulates HCV replication. Replication of HCV subgenomic replicon RNA in Huh-7 cells is inhibited by a PPAR antagonist, 2-chloro-5-nitro-N-(pyridyl) benzamide (BA), or by PPARα siRNA along with a clear accumulation of lipids upon treatment with BA [56]. On the other hand, PPARα agonist decreased HCV replication in Huh-7 cell line [46]. Similarly, in a pilot study, 15 patients received daily oral treatment with the PPARα agonist bezafibrate for 8 weeks. Plasma viral load was significantly reduced, specially the low-density viral population [57]. In addition, the development of liver steatosis in transgenic mice expressing HCV core protein requires the activation of PPARα by fibrates [58].

FXR is another NR whose activity regulates HCV replication. Bile acids, the FXR natural ligands, and synthetic agonists, enhance HCV RNA replication in an FXR-dependant manner while guggulsterone, an FXR natural antagonist, inhibits viral expression [59]. The mechanism of action of FXR on HCV cycle remains unclear. In particular, down-regulation of PPARα and upregulation of SREBP seems to be a hallmark of hepatitis C, whereas FXR increases PPARα and decreases SREBP expression. FXR dependent activation of the SHP co-repressor that inhibits the expression of many genes could have explained some of these discrepant results, but we found that SHP-siRNA did not abrogate the positive effect of FXR on HCV replication (unpublished data). Globally, FXR activation slows down glycolysis, lipogenesis, VLDL synthesis and secretion, and favors fatty acid β-oxidation in mitochondrion [19]. All these effects seem to be opposed to the global metabolic modifications of infected cells. To reconcile these discrepant data, one might consider that HCV can perturb the activity of FXR. It was recently shown that HCV NS5A protein can directly interact with FXR [60]. In cells expressing NS5A, the expression of PPARα is decreased, particularly upon FXR activation. These preliminary experiments suggest that NS5A/FXR interaction can modify the transcriptional activity of the NR leading to a re-programmed FXR activity setting the cell in a metabolic status favoring viral replication.

Conclusions and perspectives

These recent data provide compelling evidences that hepatitis viruses replication depends on metabolic factors. The expression of these factors in organs like the liver and the intestine, that regulate the host metabolic homeostasis contributes to limit the tropism of these viruses to the liver. The mechanisms underlying the dependency on metabolic factors however differ dramatically between the two viruses. NRs directly bind to and modify the activity of HBV regulatory sequences. On the opposite, the action of NRs on HCV replication seems indirect by modifying the hepatocytes metabolic status. It is, thus, tempting to speculate that for, both viruses, metabolism must be oriented towards the anabolic mode to favor the availability of essential components needed for viral replication and assembly of virions. HBV may also benefit from a direct stimulation of its transcription by the same factors that turn on the anabolic program.

To fight metabolic disorders, many attempts have been made to develop NR agonists and antagonists some of them, like fibrate or glitazone, being already approved for clinical use. In this context, FXR appears as a promising target in the treatment of dyslipidemia and an array of other diseases. The search for modulators of FXR activity is very active particularly since FXR agonists have proven their efficacy and safety in primates [61]. It now appears that treatment of chronic viral hepatitises could benefit from drugs developed for treating metabolic diseases.

Conflicts of interest statement

No potential conflict of interest was reported.

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Role of nuclear receptors in hepatitis B and C infections


