Interplay and Recruitment of FXR and HBx on cccDNA and Role on Viral Transcription

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CONTEXT

Entry bile acids (BA) receptor, NTCP, is the first line of evidence that their is a link between BA metabolism and Hepatitits B virus (HBV) replication in hepatocytes. Then, viral replication modulates gene expression that are involved in BA matabolism and especially Farnesoid X (FXR) nuclear receptor. This nuclear receptor actively contributes to BA homeostasis in liver and intestine. Finally, FXR ligands modulate its activity and protein expression. They affect completion of HBV minichromosome but also viral replication in hepatocytes. Eliminate cccDNA or silence viral transcription is the first step to a functional cure.

OBJECTIVES

Many evidences show that bile acids (BA) nuclear receptor, FXR, is a proviral factor for HBV replication. It directly links on FXR response elements located on HBV EN2/Cp promoter (1). Its ligands have a demonstrated antiviral activity against HBV replication (2). It has also been proved that FXR is important for cccDNA completion and transcription of newly viral matrix (3). In addition, it has been suggested that murine FXR interacts with the viral transcriptional regulator HBx (4). We further aim to decipher the relationship and the interaction between FXR and HBx to better understand how they can both interfere with viral transcription.

MATERIELS & METHODS

Cells:

- 293T (human embryonic kidney cell line)
- Huh7 (human hepatocarcinoma cell line)
- Differentiated HepaRG (dHepaRG)
- Viral infection: HBV WT (from HepaD38), HBV ΔHBx (from HepG2

Experiments:

- Chromatin immunoprecipitation (ChIP)
- qPCR
- Western Blot
- Nanoluc two hybrid (N2H) (5)

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RESULTS

Figure 1: FXR interacts with HBx and the AF1 domain is not necessary for this interaction. We took advantages of the Nanoluc two hybrid assay (5) to investigate the interaction between FXR and HBx. NLR values are calculated with relative Luciferase units detected from FXR (full-length and several domains) and HBx compared to Luciferase units of related controls. Interaction is positive if NLR >1.

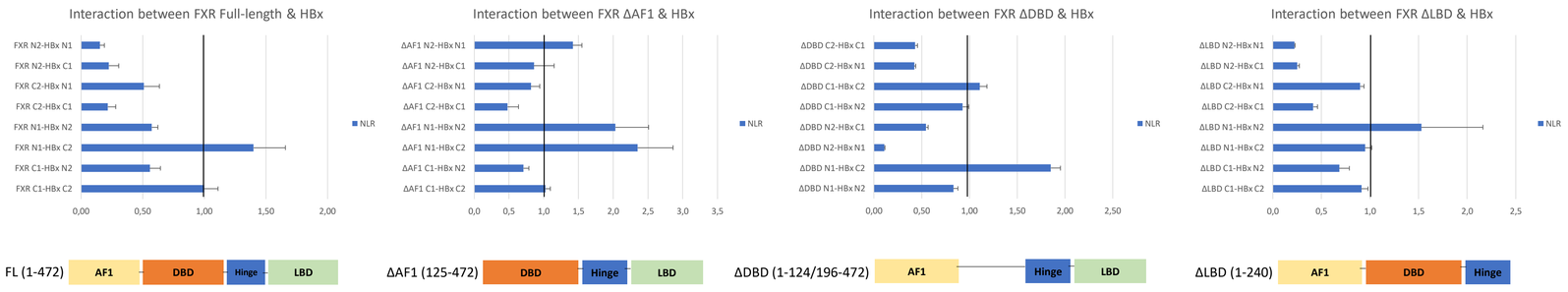


Figure 2: In Huh7, GW4064 releases FXR from EN2/Cp promoter but stabilizes it on BSEP promoter. We performed ChIP experiments in Huh7 transfected with EN2/Cp promoter plasmid. Immunoprecipitation were performed with H3K4me3 and FXR antibodies. Regions inside promoters were analyzed by qPCR.

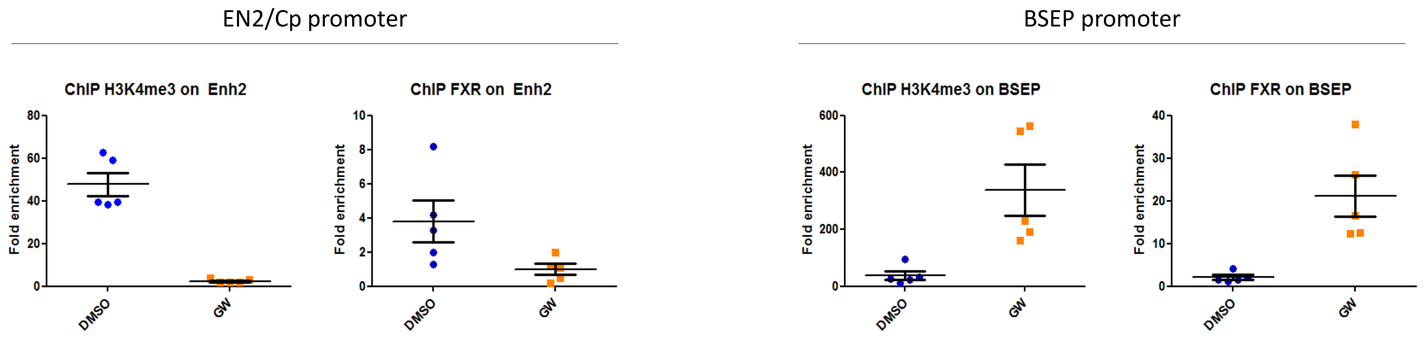


Figure 3: In relevant hepatocytes model, dHepaRG, GW4064 decreases FXR at protein and mRNA level. It as for consequences to diminish viral transcription in HBV WT infected cells. When dHepaRG were treated with GW4064 during 72h, FXR is downregulated at protein and mRNA level. Viral transcription is decreased but BSEP is still strongly upregulated.

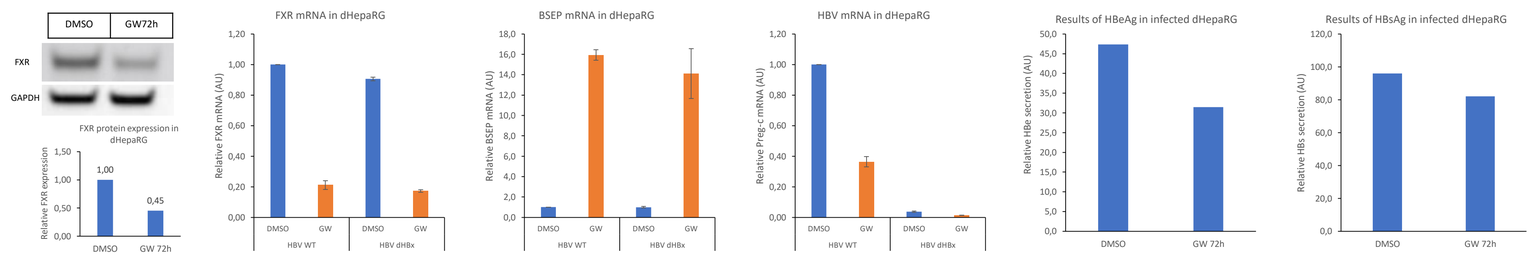
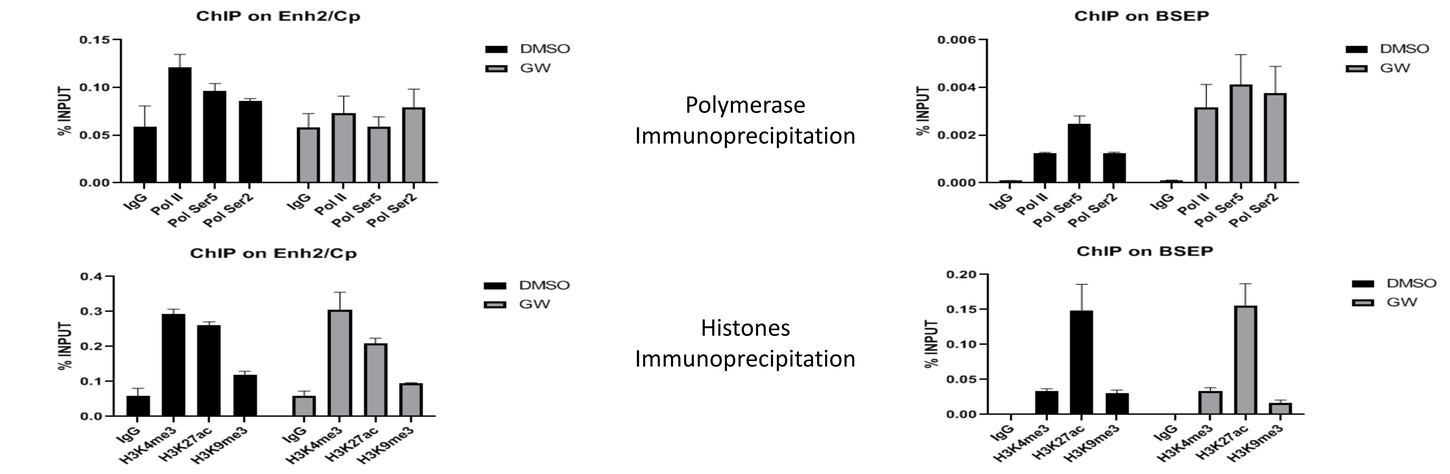


Figure 4: GW4064 decreases RNA Polymerase II recruitment at EN2/Cp promoter but increases its recruitment on BSEP promoter. We performed ChIP experiments in dHepaRG infected with HBV WT. Immunoprecipitation were performed with several histones marks and polymerase forms antibodies. Regions inside promoters were analyzed by qPCR.



CONCLUSIONS

The data suggest that:

- proviral activity of FXR depends on the fixation of FXR on EN2/Cp promoter. Also on the fact that FXR interacts with HBx and by the way could bring HBx near the EN2/Cp promoter to regulate viral transcription.

- antiviral activity of GW4064 relies on three actions from the ligand. The first is the downregulation of FXR protein and mRNA level in hepatocytes. The second is the releasing of FXR from EN2/Cp. Degrade or release FXR from the viral promoter could get away HBx from this regulated area and by consequence blocks HBx action. The last is the decrease of RNA Pol II recruitment on EN2/Cp promoter.

GW4064 is a strong HBV transcriptional inhibitor.