Recruitment of HBx on HBV DNA depends on FXR and is inhibited by FXR agonist

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1 INTRODUCTION

Bile Acids (BA) metabolism interferes with HBV replication at several steps:

1) HBV enters hepatocytes through the BA transporter NTCP (1)(2)
2) chronic hepatitis B is associated with increased liver expression of FXR, the BA nuclear receptor, and decreased expression of cellular genes under its regulation (3)
3) FXR is a proviral factor, favouring both cccDNA formation and its transcription. Conversely, FXR activation by ligands reduces cccDNA formation and transcription. In mice infected by a recombinant adeno-associated virus-2/8-HBV, FXR ligand repressed HBV DNA and HBsAg production (4).

FXR might thus be envisioned as a cellular target in anti-HBV therapy.

2 AIM

In this study, we aimed at deciphering the molecular mechanisms by which sustained FXR agonism represses the pro-transcriptional activity of FXR on HBV cccDNA transcription.

Previous data showed:
- FXR binds directly on HBV Enh2/Cp promoter region on FXR response elements (FXRE) (5).
- FXR silencing decreases cccDNA formation, completion and transcription (4).
- FXR interacts with HBV principal transcription regulator, the viral protein HBx (6).

We aim to decipher the crosstalk between FXR and HBx during transcription.

3 METHOD

Cells:
- Huh7 cell line and differentiated HepaRG (dHepaRG).

Viruses:
- HBV WT and HBV ΔHBx
- Lentiviruses expressing ShRNA against FXRα.

Experiments:
- Chromatin immunoprecipitation (ChIP)
- Antibody against H3K4me3 and H3K27ac (positive transcription marks). Antibody against FXRα.
- Transfection of a plasmid containing HBV Enh2/Cp region.

4 RESULTS

Figure 1: FXR directly binds FXRE on Enh2/Cp region and on cellular promoters. GW releases FXR from viral promoter but increases FXR presence on cellular genes.

ChIP experiments were performed on endogenous FXR in Huh7 cell line transfected with Enh2/Cp region.

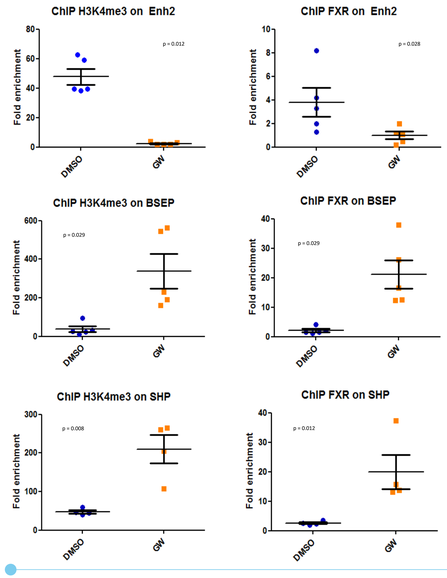


Figure 2: FXR seems to be necessary for optimal recruitment of HBx on ENh2/Cp region.

ChIP experiments were performed in Huh7 or Huh7-ShFXR co-transfected with HBV ENh2/Cp and HBx.

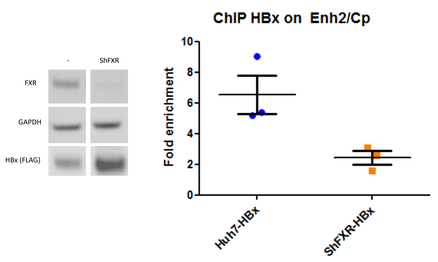


Figure 3: Short treatment with agonist transiently enhances pro-transcriptional activity of FXR.

ChIP experiments were performed in dHepaRG infected with HBV and treated 7h with GW at day 7 p.i.

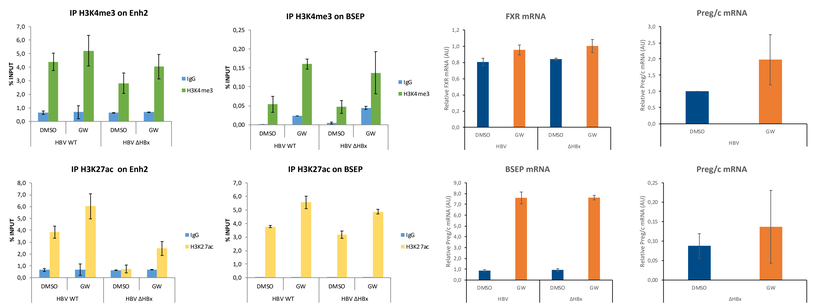


Figure 4: Sustained FXR agonism represses FXR expression and has antiviral activity.

WB and qPCR for FXR protein and mRNA expression at day 10 post-infection of dHepaRG and 72h GW treatment (days 7-10 p.i.). Preg/c mRNA were quantified by qPCR.

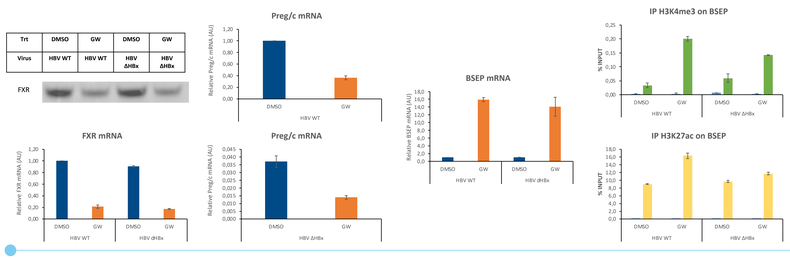
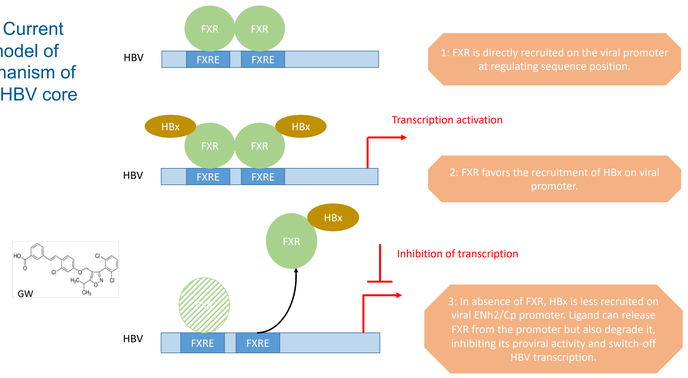


Figure 5: Current working model of FXR mechanism of action on HBV core promoter.



5 CONCLUSIONS

Altogether, the data suggest that FXR proviral activity depends on its binding to the viral genome at the Enh2/Cp region. Presence of FXR might recruit cellular factors for cccDNA completion and HBx for efficient transcription. Inhibition of FXR proviral activity by agonist might result from repression of FXR expression and from the release of FXR from its viral targets. This later effect seems to be DNA sequence dependent since agonist stabilize FXR on cellular promoters.

6 ACKNOWLEDGEMENTS

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7 REFERENCES

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8 CONTACT

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