RNA Interference Screen Identified Farnesoid X Receptor (FXR) as a Host Dependency Factor for HBV Establishment in Primary Human Hepatocytes

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Kona, Hawaii
I am an employee at Gilead Sciences Inc., USA
Need to find new target for HBV cure

A variety of host proteins have been reported as restriction or dependency factors for hepatitis B virus (HBV)

Majority of these factors were identified in transformed cell lines

We performed a focused siRNA screen in primary human hepatocytes (PHH) to evaluate the role of these factors in the HBV replication cycle
Study Design and Hit Selection Criteria

- Evaluated host factors (n=73) previously identified as restriction or dependency factors for HBV (n= 60) or other viruses (e.g. HIV, HCV)
- n=2 PHH donors; n=3 siRNA/protein; controls: siCtrl, siNTCP and siHBV
- Hit selection criteria:
  - ≥25% change in HBV DNA, HBsAg or HBeAg and ≥80% cell viability
  - Activity observed for ≥2 siRNA
Farnesoid X Receptor (FXR) Identified as Host Dependency Factor for HBV Replication in PHH

PHH Donor 1

- HBsAg: 7
- HBeAg: 1
- FXR: 0
- HBV DNA: 1

PHH Donor 2

- HBsAg: 6
- HBeAg: 3
- FXR: 1
- HBV DNA: 1

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[Diagram showing the relationship between HBsAg, HBeAg, FXR, and HBV DNA for PHH Donor 1 and PHH Donor 2]
FXR is a Transcriptional Regulator of Hepatic Bile Acid, Glucose and Lipid Metabolism

- Transcription factor belonging to the nuclear receptor superfamily
- Highly expressed in liver, intestine and kidney\(^1\)
- Functions as a bile acid (BA) sensor and regulates genes involved in bile acid homeostasis in liver\(^1\)
- Also regulates hepatic glucose and lipid metabolism\(^1\)
- FXR null mice were shown to have elevated serum BA, cholesterol and triglycerides\(^2\)

Knockdown of FXR Inhibited HBV DNA and Antigens

Mean ± SD from n=2 independent PHH donors
FXR is Required for an Early Step in Replication Cycle

Mean ± SD from n=2 independent PHH donors
Knockdown of FXR Did Not Alter NTCP Expression

qRT-PCR

Western Blot

FXR
NTCP
GAPDH

% No siRNA

siCtrl
siFXR

None
siCtrl
siFXR
Summary

- Knockdown of the majority of genes (n=52, 71%) either did not impact HBV infection or substantially reduced cell viability
- Knockdown of 19 genes inhibited at least one viral endpoint
- Nuclear bile receptor FXR identified as a host dependency factor
  - Involved in early step(s) of HBV replication cycle
  - Knock-down did not substantially alter hepatocyte viability and differentiation status
  - Studies ongoing to elucidate the MOA by which FXR influences HBV
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