Alpha-interferon induces epigenetic modification of hepatitis B virus covalently closed circular DNA in cell specific manner

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BACKGROUND

DNA methylation and histone modification

Methylated DNA

NURD Complex

Sin3A Complex

Highly Condensed & Stable Deacetylated Chromatin Structure

Transcriptional Repression
BACKGROUND

DNA methylation in mammals

Chen Z, and Riggs A D J. Biol. Chem. 2011;286:18347-18353
BACKGROUND

Natural History of Chronic HBV Infection

Infection

BACKGROUND

HBV cccDNA persists after clearance of HBsAg
BACKGROUND

Natural History of Chronic HBV Infection

Epigenetic modification of HBV cccDNA?
BACKGROUND
Histone acetylation controls HBV cccDNA activity

A. ChIP

B. HBV copies/ml of serum

<table>
<thead>
<tr>
<th>Patients</th>
<th>Input</th>
<th>IgG</th>
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<tbody>
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<td>10</td>
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</table>

\[ \text{AcH3, AcH4, HDAc1} \]

\[ \text{HBV copies/ml of serum (Log}^{10}\text{)} \]

\[ (4) \quad (4) \quad (2) \]

\[ \text{AcH3: +, -} \text{ AcH4: +, -} \text{ HDAc1: -, +} \]
HBV cccDNA is methylated in CHB

BACKGROUND

BACKGROUND

\[ r = -0.689 \]
\[ p = 0.013 \]
BACKGROUND

Interferon-alpha in CHB

- IFN-α may induce durable HBV suppression in HBeAg-positive CHB

- Mechanisms of action: ?

- Hypothesis: IFN-α induces methylation on HBV cccDNA
**METHODS**

**In vitro HBV replication system**

<table>
<thead>
<tr>
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<th>HepG2</th>
<th>Huh7</th>
<th>PH5CH8</th>
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<tr>
<td>origin</td>
<td>hepatoblastoma</td>
<td>W/D HCC cells</td>
<td>Immortalized normal human liver cells</td>
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- pHY92 plasmid: x1.1 HBV genome with CMV promoter
- HepAD38 cells: HepG2 cells stably expressing HBV pgRNA
- Pegintron: 16 ng/mL
## RESULTS

Spontaneous methylation of HBV DNA in HepG2 cells
RESULTS

IFN-α induces methylation of HBV cccDNA in cell-specific manner
RESULTS

IFN-α induces different HBV replication kinetics

IFN-α suppresses viral productivity of HBV cccDNA in HepG2 only
RESULTS

DNMT3a expression is maintained in HepG2 but suppressed in PH5CH8 during IFN-α treatment.

DNMT1

DNMT3a

DNMT3b
RESULTS

DNMT3a binds HBVcccDNA in HepG2, but not in PH5CH8

Chromatin immunoprecipitation (ChIP) assay
### SUMMARY & CONCLUSIONS

**Interferon-α in HBV cell culture model**

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<td><strong>Origin</strong></td>
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<tr>
<td><strong>IFN-α induced methylation</strong></td>
<td>↑</td>
<td>↓ →</td>
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<tr>
<td><strong>Baseline DNMT3a expression</strong></td>
<td>high</td>
<td>low</td>
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<tr>
<td><strong>DNMT3a expression after IFN-α</strong></td>
<td>maintained</td>
<td>suppressed</td>
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<tr>
<td><strong>DNMT3a binding to HBV cccDNA</strong></td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td><strong>Viral productivity after IFN-α</strong></td>
<td>↓</td>
<td>→</td>
</tr>
</tbody>
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- Anti-HBV effect of IFN-α is associated with increased HBV DNA methylation in HepG2 cell line
- IFN-α-induced antiviral effect and augmented HBV DNA methylation is not observed in human liver cells of non-neoplastic origin *in vitro*